

UNITED STATES AIR FORCE IERA

Sensory Irritation Study in Mice: JP-4, JP-8, JP-8+100

Frederick T. Whitman

ExxonMobil Biomedical Sciences, Incorporated
Laboratory Operations
1545 Route 22 East, P. O. Box 971
Annadale, NJ 08801

John P. Hinz

DISTRIBUTION STATEMENT A
Approved for Public Release
Distribution Unlimited

August 2001

20020118 253

*Approved for public release;
distribution is unlimited.*

Air Force Institute for Environment, Safety
and Occupational Health Risk Analysis
Risk Analysis Directorate
Risk Assessment Division
2513 Kennedy Circle
Brooks Air Force Base TX 78235-5116

NOTICES

When Government drawings, specifications, or other data are used for any purpose other than in connection with a definitely Government-related procurement, the United States Government incurs no responsibility or any obligation whatsoever. The fact that the Government may have formulated or in any way supplied the said drawings, specifications, or other data, is not to be regarded by implication, or otherwise in any manner construed, as licensing the holder or any other person or corporation; or as conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

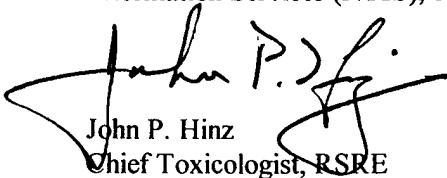
The mention of trade names or commercial products in this publication is for illustration purposes and does not constitute endorsement or recommendation for use by the United States Air Force.

The Office of Public Affairs has reviewed this report, and it is releasable to the National Technical Information Service, where it will be available to the general public, including foreign nationals.

This report has been reviewed and is approved for publication.

Government agencies and their contractors registered with Defense Technical Information Center (DTIC) should direct requests for copies to: Defense Technical Information Center, 8725 John J. Kingman Rd., STE 0944, Ft. Belvoir, VA 22060-6218.

Non-Government agencies may purchase copies of this report from: National Technical Information Services (NTIS), 5285 Port Royal Road, Springfield, VA 22161-2103.



John P. Hinz
Chief Toxicologist, RSRE



Kenneth L. Cox, Lt Col. USAF, MC, SFS

REPORT DOCUMENTATION PAGE			Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.				
1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE 31 August 2001		3. REPORT TYPE AND DATES COVERED
4. TITLE AND SUBTITLE Sensory Irritation Study in Mice: JP-4, JP-8, JP-8 + 100			5. FUNDING NUMBERS F41624-00-C-9007	
6. AUTHOR(S) Whitman, Fredrick T. Hinz, John P.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) ExxonMobil Biomedical Sciences, Inc. Laboratory Operations 1545 Route 22 East (Post Office Box-971) Annandale, NJ 08801			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Air Force Institute for Environment, Safety, and Occupational Health Risk Analysis Risk Analysis Directorate Risk Analysis Division 2513 Kennedy Circle Brooks AFB, TX 78235			10. SPONSORING/MONITORING AGENCY REPORT NUMBER IERA-RS-BR-SR-2001-0005	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION AVAILABILITY STATEMENT Approved for public release; distribution unlimited			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 words) AFIERA, in concert with Army and Navy colleagues, designed a study based on ASTM's "Standard Method E 981-84" to characterize and compare the relative potency of three jet fuels to cause respiratory tract sensory irritation. These fuels were JP-4, JP-8 and JP-8 + 100. These fuels were administered for 30 minute periods by means of a head-only exposure system to groups of four male Swiss-Webster mice. Test atmospheres laden with these fuels were presented as vapor-only (JP-4) or as a vapor/aerosol mixtures (JP-8, JP-8 + 100). Analytical sampling data revealed differences in the distribution and relative proportions of the hydrocarbon species contained in the vapor and aerosol phases. Generally, compounds with carbon numbers in the range of C11-C12 represented the principle constituents in the aerosol phase. Each fuel was tested over a range of air concentrations (685 - 11,430 mg/m3 for JP-4, 681 - 3,565 mg/m3 for JP-8, and 777 - 2,356 mg/m3 for JP-8 + 100) that resulted in minimal to severe decreases in respiratory rate. All three fuels evoked breathing patterns that were characteristic of upper airway sensory irritation at all exposure levels. Within the context of this study, there was no apparent evidence of pulmonary (deep lung) irritation or narcosis at any exposure level. The concentration that reduced the respiratory rate by 50% (RD50) was calculated for each fuel: JP-4 = 4842 mg/m3; JP-8 = 2876 mg/m3; JP-8 + 100 = 1629 mg/m3. The relative irritancy of these fuels may be ranked as follows: JP-8 + 100 > JP-8 > JP-4. Based on Alarie's 3% "rule of thumb" and without the introduction of additional information, the data suggest the following prospective PELs for these fuels: JP-4 at ~145 mg/m3; JP-8 at ~86 mg/m3; JP-8 + 100 at ~49 mg/m3.				
14. SUBJECT TERMS Upper respiratory tract, Sensory irritation, Alarie study, JP-4, JP-8, JP-8 + 100, Vapor exposures, Aerosol exposures, Mixed atmosphere exposures			15. NUMBER OF PAGES 70	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT UL	

THIS PAGE INTENTIONALLY LEFT BLANK

ExxonMobil BIOMEDICAL SCIENCES, INC.

FINAL REPORT

PROJECT NUMBER: 162951

TEST SUBSTANCES:

**JP-4 (MRD-00-629)
JP-8 (MRD-00-630)
JP-8+100 (MRD-00-631)**

SENSORY IRRITATION STUDY IN MICE

PERFORMED FOR:

**Department of the Air Force
311th Human Systems Wing
Environmental Science Branch
Brooks Air Force Base
Texas, 78235-5123**

PERFORMED AT:

**EXXONMOBIL BIOMEDICAL SCIENCES, INC.
LABORATORY OPERATIONS
1545 ROUTE 22 EAST, P.O. BOX 971
ANNANDALE, NJ 08801-0971**

**COMPLETION DATE: August 29, 2001
01TP 92**

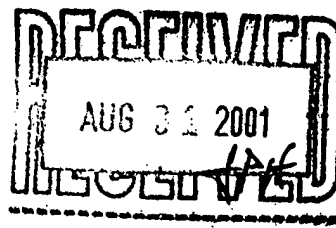


TABLE OF CONTENTS

	PAGE
APPROVAL SIGNATURES.....	4
QUALITY ASSURANCE STATEMENT	5
PERSONNEL	6
SUMMARY	7
INTRODUCTION.....	8-9
MATERIALS AND METHODS	
TEST SUBSTANCE	10
TEST SYSTEM	11-12
EXPERIMENTAL DESIGN	13-17
RESULTS	18-20
CONCLUSION	21
PROTOCOL EXCEPTIONS	21
REFERENCES	22
<u>FIGURES</u>	
FIGURE 1 SCHEMATIC OF GENERATION AND EXPOSURE SYSTEM (VAPOR)	23
FIGURE 2 SCHEMATIC OF GENERATION AND EXPOSURE SYSTEM (AEROSOL)	24
FIGURE 3 GROUP MEAN RESPIRATORY RATES JP4	29
FIGURE 4 GROUP MEAN RESPIRATORY RATES JP8	30
FIGURE 5 GROUP MEAN RESPIRATORY RATES JP8 +100.....	31
FIGURE 6 INDIVIDUAL RESPIRATORY RATES JP4.....	32
FIGURE 7 INDIVIDUAL RESPIRATORY RATES JP8.....	33
FIGURE 8 INDIVIDUAL RESPIRATORY RATES JP8.....	34
FIGURE 9 INDIVIDUAL RESPIRATORY RATES JP8 + 100.....	35
FIGURE 10 RD50 CALCULATION: JP-4, JP-8, JP-8+100.....	36

TABLE OF CONTENTS (CONT'D)

TABLES

TABLE 1	SUMMARY OF MEAN STUDY DATA	25
TABLE 2	SUMMARY OF ANIMAL RESPONSE DATA JP4	26
TABLE 3	SUMMARY OF ANIMAL RESPONSE DATA JP8	27
TABLE 4	SUMMARY OF ANIMAL RESPONSE DATA JP8 + 100.....	28
TABLE 5	SUMMARY OF ANALYTICAL DATA JP-4	37
TABLE 6	SUMMARY OF ANALYTICAL DATA JP-8	38
TABLE 7	SUMMARY OF ANALYTICAL DATA JP-8+100.....	39

APPENDIX A:

TABLES

TABLE 1A	ANALYTICAL PROCEDURES AND DETAILED HYDROCARBON ANALYSES	40-41
TABLE 2A	INDIVIDUAL HYDROCARBON DATA JP-4	42
TABLE 3A	INDIVIDUAL HYDROCARBON DATA JP-8	43
TABLE 4A	INDIVIDUAL HYDROCARBON DATA JP-8+100.....	44
TABLE 5A	INDIVIDUAL HYDROCARBON DATA PARTICLE SIZE SAMPLES	45
TABLE 6A	INDIVIDUAL HYDROCARBON DATA NEAT TEST SUBSTANCES	46

FIGURES

FIGURE 1A	CHROMATOGRAM FOR NEAT SUBSTANCE JP-4	47
FIGURE 2A	CHROMATOGRAM FOR NEAT SUBSTANCE JP-8	48
FIGURE 3A	CHROMATOGRAM FOR NEAT SUBSTANCE JP-8+100..	49
FIGURE 4A-7A	SELECTED CHROMATOGRAMS JP-4	50-53
FIGURE 8A-12A	SELECTED CHROMATOGRAMS JP-8	54-58
FIGURE 13A-15A	SELECTED CHROMATOGRAMS JP-8+100.....	59-61

APPENDIX B:

(ADDITIONAL EXPOSURES REPEATED DUE TO MISSING OR INCORRECT DATA)

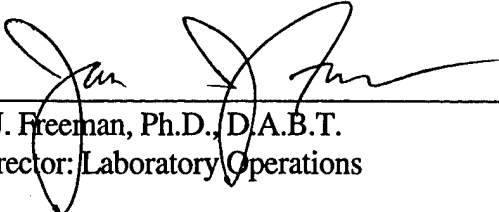
TABLES

TABLE 1B	SUMMARY OF ANALYTICAL DATA.....	62
TABLE 2B	SUMMARY OF ANIMAL RESPONSE DATA	63

FIGURES

FIGURE 1B	INDIVIDUAL RESPIRATORY RATES	64
-----------	------------------------------------	----

APPROVAL SIGNATURES

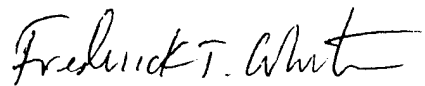


J. J. Freeman, Ph.D., D.A.B.T.
Director: Laboratory Operations

29 Aug 01
Date

I hereby accept responsibility for the validity of these data and declare that to the best of my knowledge, the study contained herein was performed under my supervision in compliance with OECD Principles of Good Laboratory Practice except as follows:

The EMBSI Industrial Hygiene Analytical Service Laboratory (IHASL) is not a fully GLP compliant laboratory, although it is accredited by the American Industrial Hygiene Association.



Frederick T. Whitman, M.P.H.
Study Director
ExxonMobil Biomedical Sciences, Inc.
1545 Route 22 East, P.O. Box 971
Annandale, New Jersey 08801-0971

29 AUG 01
Date

QUALITY ASSURANCE STATEMENT

STUDY NUMBER: 162951

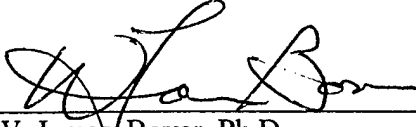
TEST SUBSTANCE: MRD-00-629, MRD-00-630, MRD-00-631

STUDY SPONSOR: Department of the Air Force

Listed below are the inspections performed by the Quality Assurance Unit of ExxonMobil Biomedical Sciences, Inc., the date(s) of inspection, and the date(s) findings were reported to the Study Director and Management.

<u>Study Phase Inspected</u>	<u>Date(s) of Inspection</u>	<u>Reported to Study Director</u>	<u>Reported to Management</u>
Protocol	06 Oct 00	06 Oct 00	13 Oct 00
Dosing (Day 0)	01 Dec 00	01 Dec 00	12,13 Dec 00
Final Report	05 May 01 to 28 Jun 01	28 Jun 01	28 Jun 01 & 16 Jul 01
Second Review of Final Report	10-13 Jul 01	13 Jul 01	13 Jul 01 & 14 Aug 01
Third Review of Final Report	13 Aug 01	13 Aug 01	13,16 Aug 01

The final report accurately reflects the methods, procedures and observations documented in the raw data.



W. James Bover, Ph.D.
Quality Assurance Section Head

29 Aug 01
Date

PERSONNEL

Study Director:	F. T. Whitman, M.P.H. ExxonMobil Biomedical Sciences, Inc. 1545 Route 22 East, P.O. Box 971 Annandale, New Jersey 08801-0971
Sponsor Representative:	John P. Hinz, M.S.
Sponsor:	Department of the Air Force 311th Human Systems Wing Environmental Science Branch Brooks Air Force Base Texas, 78235-5123
Director: Laboratory Operations:	J. J. Freeman, Ph.D., D.A.B.T.
General Toxicology Group Head:	G. W. Trimmer, B.A.
Industrial Hygiene Laboratory Supervisor	J. L. Martin, Ph.D.
Compound Preparation Supervisor:	E. J. Febbo, M.S.
Quality Assurance/Archives Section Head:	W. J. Bover, Ph.D.
Veterinarian:	R. L. Harris, D.V.M.

SUMMARY

This study was conducted to evaluate the sensory irritation potential of JP-4 (MRD-00-629), JP-8 (MRD-00-630), and JP-8 100 (MRD-00-631) in mice during inhalation exposure.

The test substances were administered head only for 30 minutes to groups of four male Swiss-Webster mice as either vapor-only (JP-4) or combined vapor/aerosol atmospheres (JP-8+100 and JP-8). Group mean exposure concentrations ranged from 685 - 11430 mg/m³ (JP-4), 681 - 3613 mg/m³ (JP-8), and 777 - 2356 mg/m³ (JP-8+100). Analytical sampling data demonstrated clear differences in the distribution and relative proportions of individual hydrocarbon species contained in the aerosol and vapor phases, with the lighter molecular weight hydrocarbons more abundant in the vapor samples. The on-line vapor and aerosol monitors demonstrated that the test atmospheres for all groups were stable throughout the exposure periods.

The three test substances evoked breathing patterns characteristic of upper airway sensory irritation at all exposure levels. Within the context and limits of this study, examination of the breathing patterns revealed no apparent pulmonary (deep lung) irritation or narcosis at any level for all three substances.

Each substance was tested over a range of air concentrations that resulted in minimal to extreme respiratory rate decreases (>50% decrease). This allowed calculation of an RD₅₀ value for each test substance. The RD₅₀ values were:

JP-4	-	4842 mg/m ³ , with 95% confidence limits of 2375 to 9873.
JP-8	-	2876 mg/m ³ , with 95% confidence limits of 2107 to 3925.
JP-8+100	-	1629 mg/m ³ , with 95% confidence limits of 1418 to 1871

Based on these results, the relative irritancy ranking of the three test substances could be described as: JP-8+100 > JP-8 > JP-4.

INTRODUCTION

This study was conducted in order to evaluate the sensory irritation potential in the mouse of JP-4 (MRD-00-629), JP-8 (MRD-00-630) and JP-8+100 (MRD-00-631).

Alarie (1966) proposed that a correlation exists between chemicals causing a decrease in respiratory rate in male Swiss-Webster-mice and sensory irritant properties in humans. Measurement of the reflex decrease in respiratory rate that occurs with stimulation of trigeminal nerve endings in the nasal mucosa of mice therefore may be used as screening tool to predict acceptable exposure concentrations or to help establish threshold limit values, short-term exposure limits, etc. The RD50, defined as the concentration associated with a 50% decrease in respiratory rate, is often used as a benchmark to evaluate airborne chemicals or mixtures of chemicals for sensory irritant effects.

The study was conducted by ExxonMobil Biomedical Sciences, Inc. (EMBSI), Laboratory Operations, Mammalian Toxicology Laboratory, 1545 Route 22 East, P.O. Box 971, Annandale, New Jersey 08801-0971 which is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC International). The analytical portion of the study was conducted by the EMBSI Industrial Hygiene Analytical Service Laboratory (IHASL), which is accredited by the American Industrial Hygiene Association.

Study Initiation (Protocol Signature Date)

November 9, 2000

Experimental Starting and Completion Date

November 29, 2000 and May 8, 2001

Justification for Selection of Test System

Swiss-Webster mice are the strain and species of choice for sensory irritation studies (ASTM E981-84, 1996).

Justification of Dosing Route

Potential human exposure may be by the inhalation route.

Compliance

This study was conducted in compliance with the following standards:

OECD, Organization for Economic Cooperation and Development, Principles of Good Laboratory Practice, C(97) 186/Final, 1997.

United States Environmental Protection Agency, 40 CFR Part 792, Toxic Substances Control Act (TSCA), Good Laboratory Practice Standards (GLP's), Final Rule 1989.

This study was conducted in general agreement with the following guidelines and standards:

Standard Test Method for Estimating Sensory Irritation of Airborne Chemicals. American Society for Testing and Materials. Designation: E981-84 (Reapproved 1996).

Animal Welfare Act of 1966 (P.L. 89-544), as amended in 1970, 1976, and 1985. Code of Federal Regulations, Title 9 [Animals and Animal Products], Subchapter A - Animal Welfare Parts 1, 2, and 3.

Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, National Academy Press, Washington, D.C., 1996.

MATERIALS AND METHODS

TEST SUBSTANCE

Substance Identification

<u>EMBSI Identification:</u>	<u>Sponsor Identification:</u>
MRD-00-629	JP-4
MRD-00-630	JP-8
MRD-00-631	JP-8+100
Supplier:	Mantech Environmental Tech Inc. WPAB Area B Bldg. 79 Rm. 154 Dayton, OH 45433
Date Received:	July 19, 2000
Expiration Date:	July 2005
Description:	Pale yellow liquids
Storage Condition:	Room temperature

Each test substance, as received, was considered the "pure" substance.

Characterization of Test Substance

Samples of the test atmosphere at each exposure level were analyzed by gas chromatography. The concentrations of selected individual components were reported. Samples of each neat (liquid) test substance also were analyzed by gas chromatography for reference and comparison to the air samples.

Analysis of Mixtures

Not applicable to this study.

Solubility

Not applicable to this study.

Sample Retention

Archival samples of the test substances were not retained for this study.

Carrier

Air

MATERIALS AND METHODS

TEST SYSTEM

Test Animal

Species:	Mouse
Strain/stock:	Swiss-Webster
Supplier:	Charles River Laboratories, Inc. Portage, MI

Animal Receipt Information

<u>Receipt Date:</u>	<u>Order Number:</u>
7 November 2000	20070958
5 December 2000	20076820
2 January 2001	20100021
7 March 2001	20114956
24 April 2001	20126876

Quarantine and Acclimation Period

At least 7 days; animals were examined for viability at least once daily.

Number and Sex/Group

4 males

Age at Initiation of Dosing

5 - 10 weeks

Weight at Initiation of Dosing

24 - 30 grams

Animal Identification

Tail tattoo and corresponding cage identification.

Selection

More animals than required for the conduct of the study were purchased and acclimated. Animals determined to be unsuitable for inclusion in the study because of poor health, outlying body weight, or other abnormalities were excluded from selection by the attending veterinarian, Study Director and/or the technical staff. Animals were allocated to study groups immediately prior to exposure on the basis of general health and body weight requirements.

Housing

Room: PE112
Housing: Single housed during the study period.
Caging: Suspended stainless steel and wire mesh with absorbent paper below cages.

Feed

PMI Certified Rodent Diet Checkers 5002

Manufacturer: PMI Feeds, Richmond, Indiana
Analysis: Performed by PMI Feeds. Copies of the feed analyses are maintained at the EMBSI Laboratory.
Contaminants: There were no known contaminants in the feed believed to have been present at levels that may have interfered with this study.

The availability of feed was checked at least once daily for all animals.

Water

Automatic Watering System, ad libitum

Supplier: ExxonMobil Research and Engineering, Potable Water System.
Analysis: Periodic analysis is the responsibility of EMBSI. A copy of the results is maintained at EMBSI.
Contaminants: There were no known contaminants in the water believed to have been present at levels that may have interfered with this study.

The availability of water was checked at least once daily for all animals.

Environmental Conditions

Temperature: 64 to 72 degrees Fahrenheit
Humidity: 30 to 70 percent relative humidity
Lighting: Approximately 12 hours light (0600 to 1800) and 12 hours dark (1800 to 0600) by automatic timer.

Monitored at least once daily. Additionally, a non-validated computerized system monitored the temperature, humidity, and lighting continuously for alarm purposes.

EXPERIMENTAL DESIGN

Preparation of Test Substance

The test substance was administered as received.

Experimental Groups

Test Substance	Mean Analytical Concentration (mg/m ³)	Number of Animals
JP - 4 (MRD-00-629)	11430 \pm 66	4
JP - 4 (MRD-00-629)	1888 \pm 55	4
JP - 4 (MRD-00-629)	956 \pm 59	4
JP - 4 (MRD-00-629)	685 \pm 56	4
JP - 8 (MRD-00-630)	3565 \pm 129	4
JP - 8 (MRD-00-630)	1837 \pm 68	4
JP - 8 (MRD-00-630)	1090 \pm 22	4
JP - 8 (MRD-00-630)	681 \pm 40	4
JP - 8 (MRD-00-630)	708 \pm 33	4
JP - 8 + 100 (MRD-00-631)	2356 \pm 10	4
JP - 8 + 100 (MRD-00-631)	1519 \pm 39	4
JP - 8 + 100 (MRD-00-631)	777 \pm 26	4

Note: Two additional groups were exposed but not presented in the main study data due to missing or inconsistent analytical samples. The results of these additional exposures are presented in Appendix B.

Administration of Test Substance

Figures 1-2 present schematic drawings of the test atmosphere generation and exposure systems.

Each test substance was administered as either an aerosol, vapor or mixed atmosphere in air.

Vapor test atmospheres were generated by using a syringe pump to deliver the test substance to the inside surface of a heated glass "counter current" generator. The vapor generator was a cylindrical glass tube impressed with a spiral indentation that served as a channel for the liquid test substance. The spiral indentation was heated with heating tape to a constant temperature (dependent on the target concentration), and continuously monitored by a thermocouple probe and digital thermometer. The test substance volatilized as it flowed down the heated generator. The resulting vapors were drawn into the exposure chamber with the supply air moving countercurrent to the liquid flow.

Alternatively, test atmospheres were generated as a liquid droplet aerosol using a Collison nebulizer. The nebulizer's reservoir was filled with the test substance to a level just below the top of the liquid feed tube. Compressed air was supplied to the nebulizer, metered by a rotameter at a rate sufficient to aerosolize the test substance. The resultant liquid aerosol atmosphere mixed with additional room air that was drawn through the reservoir and expelled into the chamber.

Concentration Determinations

Exposure concentrations were determined on both a nominal and analytical basis. The nominal concentration was calculated by dividing the net weight of the test substance used during the exposure by the total volume of air passing through the chamber.

Analytical chamber concentrations were determined three times during each exposure by drawing a known volume of chamber air, metered by a critical orifice, through a sampling train consisting of a 25 mm glass fiber filter for analysis of non-volatile aerosol followed by a charcoal sorbent tube for total volatile hydrocarbons (vapor). After sampling, the sorbent tubes were capped and the filter samples were placed in sealed glass vials containing approximately 10 ml carbon disulfide. The sorbent tubes and filters were then submitted to the Industrial Hygiene Analytical Service Laboratory (IHASL) for subsequent analysis.

Both sample types (aerosol and vapor) were analyzed by gas chromatography/FID and each reported as total hydrocarbon concentration (THC). Additionally, one sample set (filter and sorbent tube) from each exposure was analyzed for a standard

list of representative hydrocarbons in order to evaluate and compare the distribution of individual components within the aerosol and vapor phases.

The analytical exposure concentrations (THC) were calculated both separately and as the sum of the aerosol and vapor phase concentrations. One sample of each neat test substance also was analyzed for reference and comparison to the chamber samples.

An on-line infrared vapor monitor (MIRAN 1A) was used during each exposure (when practical due to the amount of aerosol present) to monitor the relative levels and stability of the vapor phase of the chamber atmosphere.

An on-line photometric particle monitor (Sibata Model P5) also was used during each exposure to monitor the relative levels and stability of the aerosol present in the chamber atmospheres.

Particle Size Analysis

A particle size determination of the aerosol portion of the test atmosphere was conducted once for each test substance, during a representative animal exposure, using a Sierra Instruments Model 210 Cascade Impactor. Preweighed glass fiber filters were used to collect the aerosol on each stage. A bulk estimation technique was employed to characterize the particle size distribution of the test atmosphere. The change in weight of the filter for each stage was measured and the cumulative percent of the sample collected on each stage was calculated. This information plus the stage constants (size cutoff diameters in microns) for the impactor were used, with the aid of a computer, to calculate the 15.9%, 50.0%, and 84.1% particle sizes (equivalent aerodynamic diameter), the geometric standard deviation, and the estimated percent of the aerosol less than or equal to 1, 10, and 15 microns in size.

Additionally, the filters from the three stages closest to the 15.9%, 50.0%, and 84.1% particle sizes (equivalent aerodynamic diameter) were analyzed by gas chromatography/FID for individual hydrocarbon concentrations.

Animal Exposure Procedures

The chamber used for the exposures was all glass and had a total volume of approximately 4.0 liters. It operated under slight negative pressure to the room at approximately 30.0 liters per minute airflow, regulated by a calibrated flow-limiting orifice. The theoretical equilibration time (T_{99}) was calculated as 36 seconds, which is approximately 2% of the exposure duration.

The test animals were loaded into body-only plethysmographs which isolated the animal's head from its body via a latex dam. The plethysmographs were then mounted onto the glass exposure chamber such that the animals received head-only exposures to the test atmosphere.

Each animal was monitored by a differential air pressure transducer that converted the tidal pressure changes produced by the animal's breathing within the plethysmograph into an electrical signal. The signals produced by the tidal pressure changes (animal breathing patterns) were recorded on an eight-channel analog oscillograph (Gould, Model RS 3800).

Each exposure group was monitored in the following sequence:

- | | |
|-----------|---|
| Pre-test: | At least 10 minutes of room air to establish baseline rates. |
| Exposure: | 30 minutes of test substance exposure. |
| Recovery: | At least 10 minutes of room air to monitor return to pre-test levels. |

Animal Observations

Individual animal observations were performed before, during and after each exposure.

Termination

All test animals were euthanized via asphyxiation with carbon dioxide after completion of the exposure and discarded without further evaluation.

Animal Response Evaluation

Animal respiratory rates and breathing patterns were determined from the oscillographic record. The average pretest rate and the lowest representative rate during exposure were determined for each animal in a group. The low rate was divided by the pretest rate to obtain a "Percent of Pretest" value. The Percent of Pretest value was subtracted from 100% to yield the animal response (Percent Change in Rate). Individual animals in each group were evaluated first, then averaged to determine the mean group response. The post-exposure (recovery) rates were similarly evaluated to determine the recovery response - the percent return to pretest rates.

Statistical Analysis

Statistical analyses included means and standard deviations for relevant study data. (Snedecor and Cochran, 1989).

The mean group responses and exposure concentrations were entered into a computerized least-squares analysis to determine the concentration of test material required to reduce respiratory rate by 50% (RD_{50}), the 95% confidence limits, the slope function of the plotted data, and the fit of the data from the experiment (Snedecor and Cochran, 1989; Litchfield and Wilcoxon, 1949).

Records

The protocol, all raw data, the final report, computer generated listings of raw data, and supporting documentation are maintained on file in the EMBSI archives. Raw data for the analytical samples will be maintained in the Industrial Hygiene Laboratory files.

RESULTS

EXPOSURE CONCENTRATIONS:

Tables 1-13 present summaries of the analytical data. Appendix A presents the analytical methods and summaries of the individual hydrocarbon analyses for selected samples.

JP-4 (MRD-00-629)

Four groups of male mice were exposed for 30 minutes to total analytical concentrations of 11430, 1888, 956, or 685 mg/m³. The exposure atmospheres for this test substance (generated as a vapor) essentially contained no aerosol except at the highest concentration (11430 mg/m³). A small amount of aerosol was found at this level (82 mg/m³) although it represented less than 1% of the total concentration.

A particle size sample was taken during the exposure at the highest level (11430 mg/m³) however there was insufficient aerosol present to accurately calculate a particle size distribution.

JP-8 (MRD-00-630)

Four groups of male mice were exposed for 30 minutes to total analytical concentrations of 3565, 1837, 1090, or 681 mg/m³. The exposures for this test substance (generated as an aerosol) were predominantly vapor although they included significant amounts of aerosol. The proportion of aerosol ranged from 3% at 681 mg/m³ to 35% at 3613 mg/m³. There was a clear difference in the distribution and relative proportions of individual hydrocarbon species contained in the aerosol and vapor phases, with the lighter molecular weight hydrocarbons more abundant in the vapor samples (see Appendix A for individual hydrocarbon analyses and chromatograms).

A fifth group of mice was exposed to a vapor-generated atmosphere (708 mg/m³) at a level similar to the lowest aerosol-generated group (681 mg/m³) in order to compare the animal responses to these potentially different exposures. This group contained no detectable aerosol.

A particle size sample was taken during the exposure at the 1090 mg/m³ level. The results of this analysis showed a median aerodynamic particle size of 0.25 microns, with 99.7% of the particles less than or equal to 10 microns and 84.7% less than or equal to 1.0 micron. This demonstrates that the aerosol was highly respirable.

JP-8+100 (MRD-00-631)

Three groups of male mice were exposed for 30 minutes to total analytical concentrations of 2356, 1519, or 777 mg/m³. The exposures for this test substance also were predominantly

vapor phase with the proportion of aerosol ranging from 26% at 2356 mg/m³ to 4% at 777 mg/m³. The difference in the relative proportions of individual hydrocarbon species between the aerosol and vapor phases was similar to JP-8, except there appeared to be an even higher amount of lighter molecular weight hydrocarbons in the vapor samples (see Appendix A for individual hydrocarbon analyses and chromatograms).

A particle size sample was taken during the exposure at the 2356 mg/m³ level. The results of this analysis showed a median aerodynamic particle size of 1.27 microns, with 99.9% of the particles less than or equal to 10 microns and 36.5% less than or equal to 1.0 micron. This demonstrates that the aerosol phase of JP-8+100 also was highly respirable.

ANIMAL RESPONSE DATA:

Tables 2-4 present summaries of the animal response data. Figures 3-9 present graphs of the individual and group mean respiratory rates.

JP-4 (MRD-00-629)

Group mean respiratory rates were decreased from baseline values 58%, 51%, 28%, and 11% at mean exposure concentrations of 11430, 1888, 956, and 685 mg/m³, respectively. Breathing patterns characteristic of sensory irritation were observed in all mice in the three highest exposure concentrations; only 2 of the 4 mice in the lowest exposure group exhibited slight irritation (2 were normal). There was no evidence of narcosis or pulmonary irritation at any level.

The mice in the two highest exposure groups (11430 and 1888 mg/m³) exhibited a decreased recovery following exposure (61% and 82% of baseline values) compared to the two lower exposure groups which returned to pre-exposure levels (103% at 956 mg/m³; 96% at 685 mg/m³).

All mice in all four groups appeared normal at clinical observations performed prior to, during, and immediately after the exposures.

JP-8 (MRD-00-630)

Group mean respiratory rates were decreased from baseline values 50%, 46%, 38%, and 22% at mean exposure concentrations of 3565, 1837, 1090, or 681 mg/m³, respectively. Breathing patterns characteristic of sensory irritation were observed in all mice in all groups. There was no evidence of narcosis or pulmonary irritation at any level.

An additional group was exposed to JP-8 as a vapor-only atmosphere at 708 mg/m³ and produced a similar response (28% decrease) compared to the aerosol-generated 681 mg/m³ group (22% decrease).

The post-exposure recovery responses were depressed in all five groups, with the amount of depression proportional to the exposure concentration (75%, 77%, 86%, 93%, and 86% of baseline values at 3565, 1837, 1090, 681, and 708 mg/m³, respectively).

All mice in all five groups appeared normal at clinical observations performed prior to, during, and immediately after the exposures.

JP-8+100 (MRD-00-631)

Group mean respiratory rates were decreased from baseline values 63%, 53%, or 18% at mean exposure concentrations of 2356, 1519, or 777 mg/m³, respectively. Breathing patterns characteristic of sensory irritation were observed in all mice in all groups. There was no evidence of narcosis or pulmonary irritation at any level.

The mice in the two highest exposure groups (2356 and 1519 mg/m³) exhibited a decreased recovery following exposure (59% and 68% of baseline values) compared to the lowest exposure group (777 mg/m³) which returned to pre-exposure levels (103%).

All mice in all three groups appeared normal at clinical observations performed prior to, during, and immediately after the exposures.

RD₅₀ CALCULATIONS:

Figure 10 presents a graph of the exposure concentrations vs. respiratory rate decreases.

The exposure concentration of each test substance that would produce a 50% decrease in respiratory rate (RD₅₀) was calculated to be:

JP-4	-	4842 mg/m ³ , with 95% confidence limits of 2375 to 9873.
JP-8	-	2876 mg/m ³ , with 95% confidence limits of 2107 to 3925.
JP-8+100	-	1629 mg/m ³ , with 95% confidence limits of 1418 to 1871

CONCLUSIONS

Exposures to test atmospheres of each of the three test substances, JP-4 (MRD-00-629), JP-8 (MRD-00-630) and JP-8+100 (MRD-00-631) produced breathing patterns characteristic of upper airway sensory irritation in mice. Within the context and limits of this study, examination of the breathing patterns revealed no apparent pulmonary (deep lung) irritation or narcosis at any level for all three substances.

The exposures were generated as vapor-only (JP-4) or mixed vapor/aerosol atmospheres (JP-8 and JP-8+100). Each substance was tested over a range of air concentrations that resulted in minimal to extreme respiratory rate decreases (>50% decrease). The calculated RD₅₀ values showed a relative irritancy ranking of JP-8+100 > JP-8 > JP-4.

Analytical sampling data demonstrated clear differences in the distribution and relative proportions of individual hydrocarbon species contained in the aerosol and vapor phases. As expected, there were differences among the three test substances with JP-4 exhibiting a range of lower molecular weight hydrocarbons compared to JP-8 or JP-8+100. Within the mixed vapor/aerosol atmospheres of JP-8 and JP-8+100, the vapor phase samples showed a greater abundance of lighter hydrocarbons compared to the aerosol phase samples. The on-line vapor and aerosol monitors demonstrated that the test atmospheres for all groups were stable throughout the exposure periods.

PROTOCOL EXCEPTIONS

On six occasions the animal room temperature or humidity was outside of the ranges specified in the protocol. These slight and transient deviations should not have affected the quality or integrity of the data.

REFERENCES

Additional references for the Alarie sensory irritation assay:

Alarie, Y. (1966) Arch. Environ. Health 13:433

Alarie, Y. (1981) Fd. Cosmet. Toxicol. 19:623

Barrow, C., Alarie, Y., Warrick, J. and Stock, M. (1977) Arch. Environ. Health 32:68

Nielsen, G.D., and Alarie, Y. (1982) Tox. Appl. Pharm. 65:459

Weyel, D.A., Rodney, B.S., and Alarie, Y. (1982) Tox. Appl. Pharm. 64:423

FIGURE 1 - SCHEMATIC OF GENERATION AND EXPOSURE SYSTEM
(COUNTER-CURRENT VAPOR GENERATOR)

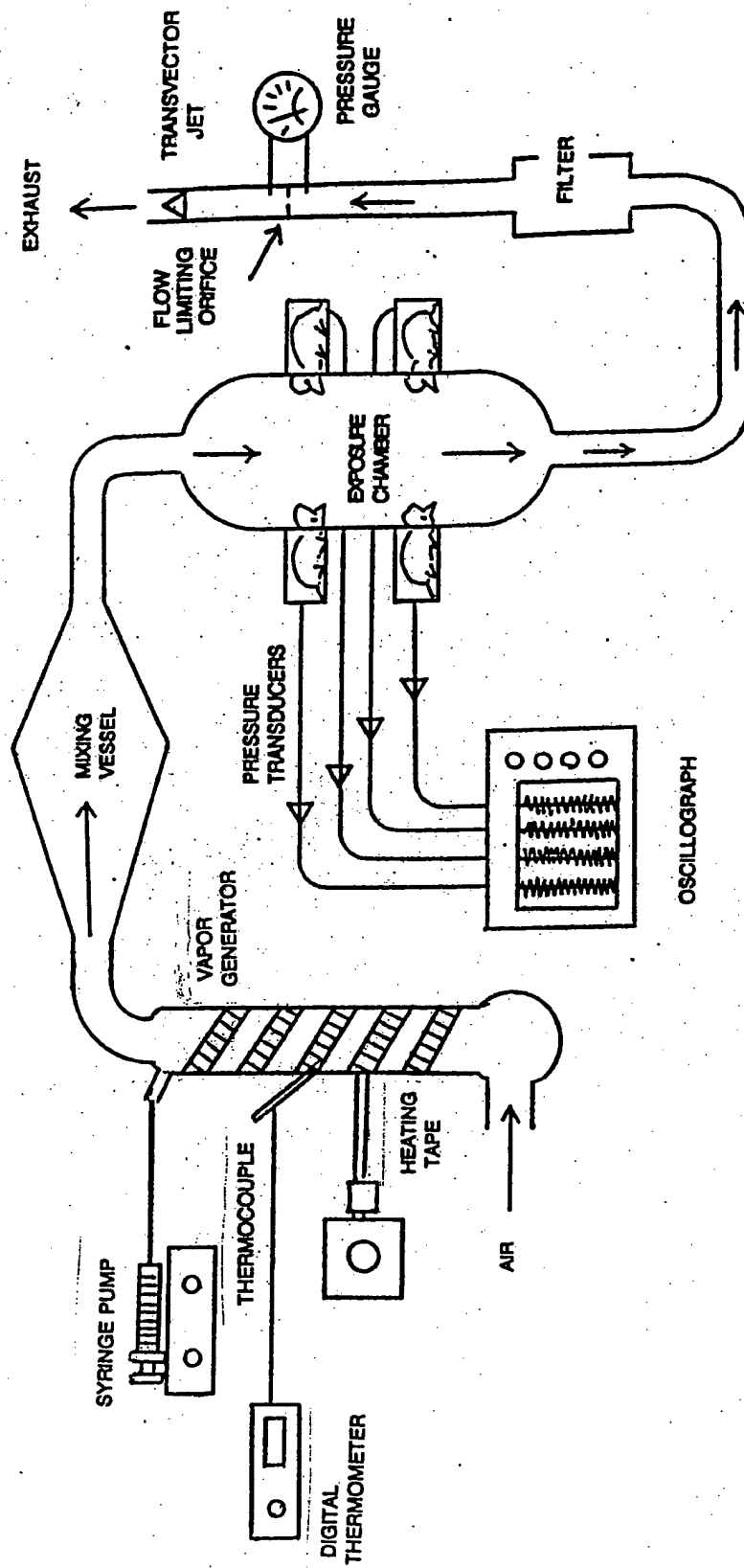


FIGURE 2 - SCHEMATIC OF GENERATION AND EXPOSURE SYSTEM
(NEBULIZER)

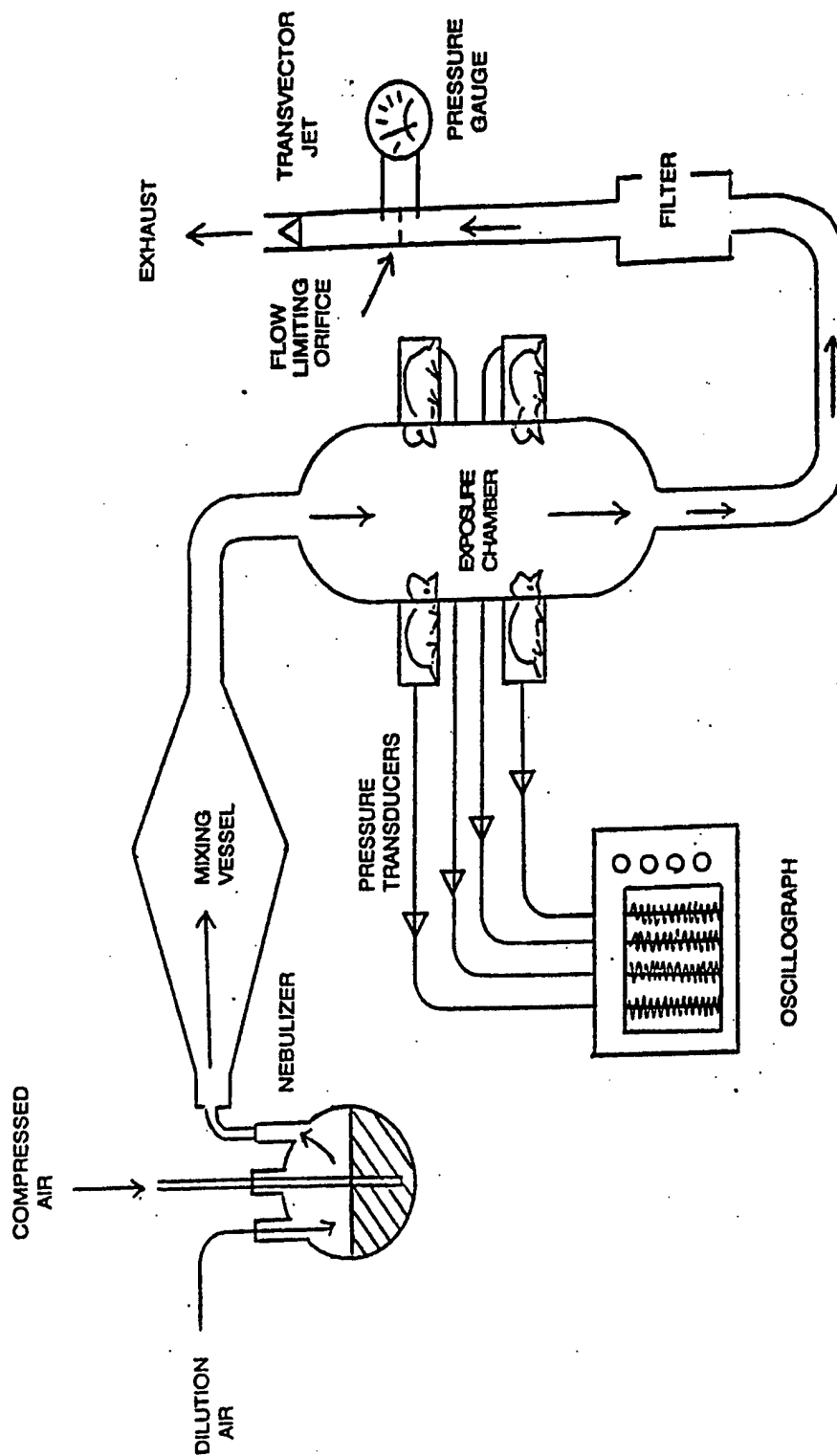


TABLE 1 - SUMMARY OF MEAN STUDY DATA

Test Substance	JP - 4				JP - 8				JP - 8 + 100			
Total Exposure Concentration:	11430	1888	956	685	3565	1837	1090	681	708	2356	1519	777
Mean Concentration (mg/m ³)	66	55	59	56	129	68	22	40	33	10	39	26
Standard Deviation	0.6	2.9	6.2	8.2	3.6	3.7	2.0	5.9	4.7	0.4	2.5	3.3
Coefficient of Variation (%)												
Vapor/Aerosol Concentration:												
Vapor Concentration (mg/m ³)	11349	1888	956	685	2335	1379	920	658	708	1754	1220	748
Aerosol Concentration (mg/m ³)	81	0	0	0	1230	459	170	23	0	603	300	29
% Aerosol	0.7	0	0	0	34.5	25.0	15.6	3.4	0	25.6	19.7	3.7
Particle Size Analysis:												
Median particle size (um)	5.35						0.25			1.27		
Geometric Standard Deviation	2.21						3.89			2.00		
Percent Less Than 10 microns	78.4						99.7			99.9		
Percent Less Than 1 micron	1.7						84.7			36.5		
Animal Response Data:												
Percent Respiratory Rate Decrease	-58	-51	-28	-11	-50	-46	-38	-22	-28	-63	-53	-18
Percent Recovery to Baseline	61	82	103	96	75	77	86	93	86	59	68	103
RD ₅₀ (mg/m ³)	4842				2876				1629			
95 % Confidence Interval	2375 - 9873				2107 - 3925				1418 - 1871			

* - Atmosphere generated as vapor only

TABLE 2 - SUMMARY OF ANIMAL RESPONSE DATA
JP-4 (MRD-00-629)

CONCENTRATION	ANIMAL NUMBER	BODY WEIGHT (g)	EXPOSURE RESPONSE (%)	RECOVERY RESPONSE (%)	IRRITATION TYPE/ SEVERITY*	GROSS OBSERVATIONS	
						(pretest) (inchamber)	(postdose)
11,430 mg/m ³	IAY933	28	-61	71	SENSORY/EXTREME	NOA	NOA
	IAY934	28	-56	57	SENSORY/EXTREME	NOA	NOA
	IAY945	29	-53	53	SENSORY/EXTREME	NOA	NOA
	IAY950	29	-61	62	SENSORY/EXTREME	NOA	NOA
	MEAN	29	-58	61			
	S.D.	0.6	3.9	7.8			
1,888 mg/m ³	IAY939	30	-45	85	SENSORY/MODERATE	NOA	NOA
	IAY941	30	-56	79	SENSORY/EXTREME	NOA	NOA
	IAY943	30	-56	76	SENSORY/EXTREME	NOA	NOA
	IAY944	30	-47	87	SENSORY/MODERATE	NOA	NOA
	MEAN	30	-51	82			
	S.D.	0.0	5.8	5.1			
956 mg/m ³	IAY952	28	-29	94	SENSORY/MODERATE	NOA	NOA
	IAY953	27	-33	98	SENSORY/MODERATE	NOA	NOA
	IAY963	29	-30	115	SENSORY/MODERATE	NOA	NOA
	IAY972	28	-18	105	SENSORY/SLIGHT	NOA	NOA
	MEAN	28	-28	103			
	S.D.	0.8	6.6	9.2			
685 mg/m ³	IAZ386	27	-12	97	SENSORY/SLIGHT	NOA	NOA
	IAZ394	28	-9	96	SENSORY/NONE	NOA	NOA
	IAZ402	27	-16	91	SENSORY/SLIGHT	NOA	NOA
	IAZ405	27	-7	100	SENSORY/NONE	NOA	NOA
	MEAN	27	-11	96			
	S.D.	0.5	3.9	3.7			

NOA - NO OBSERVABLE ABNORMALITIES

* Severity categorized as: slight = 12-19%; moderate = 20-49%; extreme = ≥ 50%.

TABLE 3 - SUMMARY OF ANIMAL RESPONSE DATA
JP-8 (MRD-00-630)

CONCENTRATION	ANIMAL NUMBER	BODY WEIGHT (g)	EXPOSURE RESPONSE (%)	RECOVERY RESPONSE (%)	IRRITATION TYPE/ SEVERITY*	GROSS OBSERVATIONS	
						(pretest)	(inchamber) (postdose)
3613 mg/m ³	IAZ385	29	-54	76	SENSORY/EXTREME	NOA	NOA
	IAZ391	29	-54	72	SENSORY/EXTREME	NOA	NOA
	IAZ397	30	-51	74	SENSORY/MODERATE	NOA	NOA
	IAZ399	29	-42	76	SENSORY/MODERATE	NOA	NOA
	MEAN	29	-50	75			
	S.D.	0.5	5.7	1.9			
1837 mg/m ³	IAY981	24	-42	80	SENSORY/MODERATE	NOA	NOA
	IAY984	24	-52	64	SENSORY/EXTREME	NOA	NOA
	IAY986	24	-54	70	SENSORY/EXTREME	NOA	NOA
	IAY989	24	-34	95	SENSORY/MODERATE	NOA	NOA
	MEAN	24	-46	77			
	S.D.	0.0	9.3	13.5			
1090 mg/m ³	IAY974	27	-41	77	SENSORY/MODERATE	NOA	NOA
	IAY976	27	-33	90	SENSORY/MODERATE	NOA	NOA
	IAY977	30	-37	90	SENSORY/MODERATE	NOA	NOA
	IAY994	27	-41	85	SENSORY/MODERATE	NOA	NOA
	MEAN	28	-38	86			
	S.D.	1.5	3.8	6.1			
681 mg/m ³	IAZ393	28	-24	91	SENSORY/MODERATE	NOA	NOA
	IAZ400	28	-25	86	SENSORY/MODERATE	NOA	NOA
	IAZ401	28	-15	100	SENSORY/SLIGHT	NOA	NOA
	IAZ403	28	-23	95	SENSORY/MODERATE	NOA	NOA
	MEAN	28	-22	93			
	S.D.	0.0	4.6	5.9			
708 mg/m ³	IAY978	28	-25	86	SENSORY/MODERATE	NOA	NOA
	IAY979	27	-36	73	SENSORY/MODERATE	NOA	NOA
	IAY980	28	-30	90	SENSORY/MODERATE	NOA	NOA
	IAY982	27	-22	96	SENSORY/MODERATE	NOA	NOA
	MEAN	28	-28	86			
	S.D.	0.6	6.1	9.7			

NOA - NO OBSERVABLE ABNORMALITIES

* Severity categorized as: slight = 12-19%; moderate = 20-49%; extreme = ≥ 50%.

TABLE 4 - SUMMARY OF ANIMAL RESPONSE DATA (CONT'D)
JP-8 + 100 (MRD-00-631)

CONCENTRATION	ANIMAL NUMBER	BODY WEIGHT (g)	EXPOSURE RESPONSE (%)	RECOVERY RESPONSE (%)	IRRITATION TYPE/ SEVERITY*	GROSS OBSERVATIONS	
						(pretest) (in-chamber)	(postdose)
2356 mg/m ³	IAZ390	28	-64	56	SENSORY/EXTREME	NOA	NOA
	IAZ392	28	-70	46	SENSORY/EXTREME	NOA	NOA
	IAZ395	29	-52	77	SENSORY/EXTREME	NOA	NOA
	IAZ404	27	-67	58	SENSORY/EXTREME	NOA	NOA
	MEAN	28	-63	59			
	S.D.	0.8	7.9	12.9			
1519 mg/m ³	IAZ459	25	-54	48	SENSORY/EXTREME	NOA	NOA
	IAZ460	26	-50	75	SENSORY/EXTREME	NOA	NOA
	IAZ467	26	-52	65	SENSORY/EXTREME	NOA	NOA
	IAZ469	26	-55	82	SENSORY/EXTREME	NOA	NOA
	MEAN	26	-53	68			
	S.D.	0.5	2.2	14.8			
777 mg/m ³	IAZ466	27	-17	98	SENSORY/SLIGHT	NOA	NOA
	IAZ468	27	-16	116	SENSORY/SLIGHT	NOA	NOA
	IAZ470	26	-15	103	SENSORY/SLIGHT	NOA	NOA
	IAZ471	26	-22	94	SENSORY/MODERATE	NOA	NOA
	MEAN	27	-18	103			
	S.D.	0.6	3.1	9.6			

NOA - NO OBSERVABLE ABNORMALITIES

* Severity categorized as: slight = 12-19%; moderate = 20-49%; extreme = ≥ 50%.

FIGURE 3 - GROUP MEAN RESPIRATORY RATES: JP-4 (MRD-00-629)

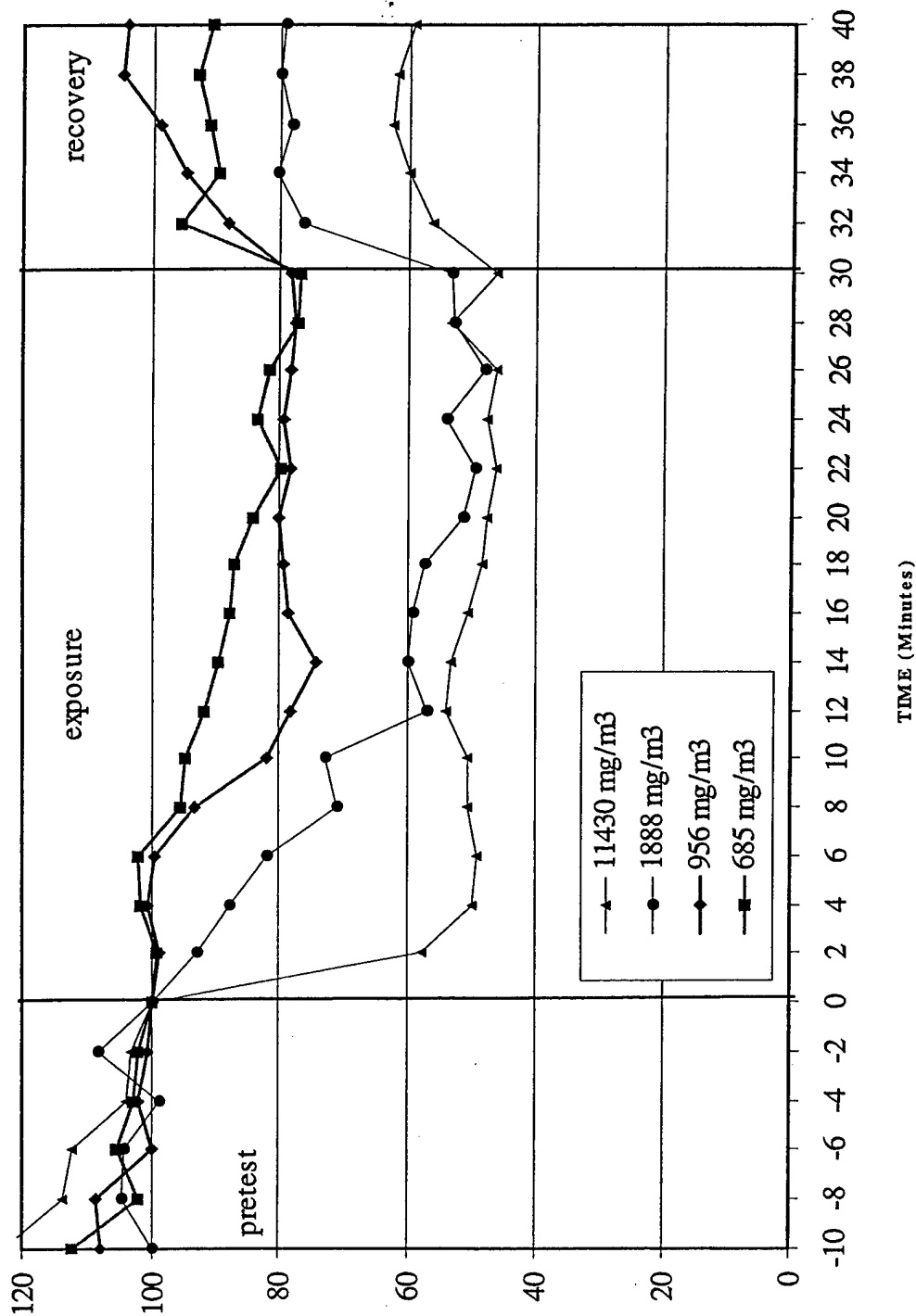


FIGURE 4 - GROUP MEAN RESPIRATORY RATES: JP-8 (MRD-00-630)

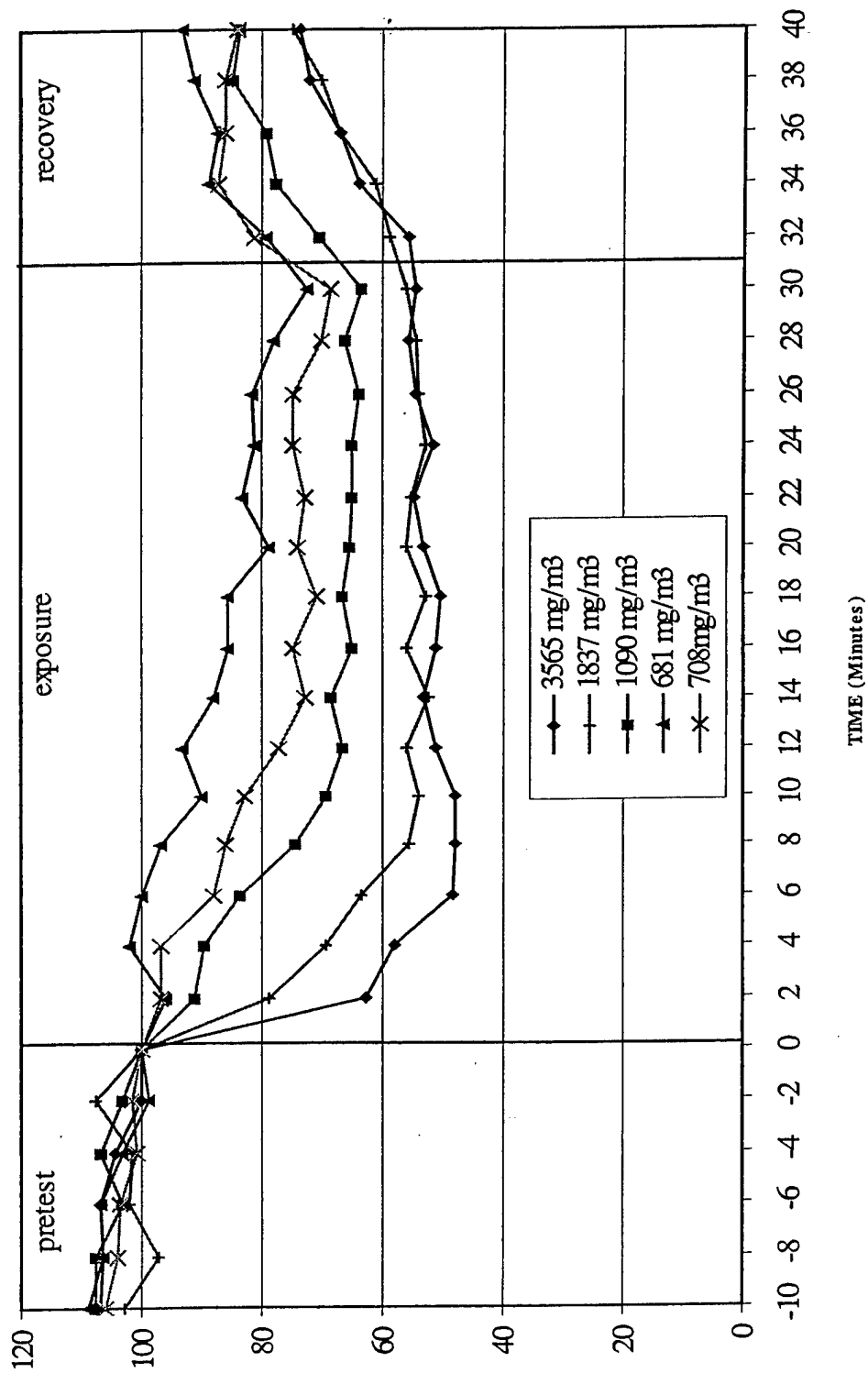


FIGURE 5 - GROUP MEAN RESPIRATORY RATES: JP-8+100 (MRD-00-631)

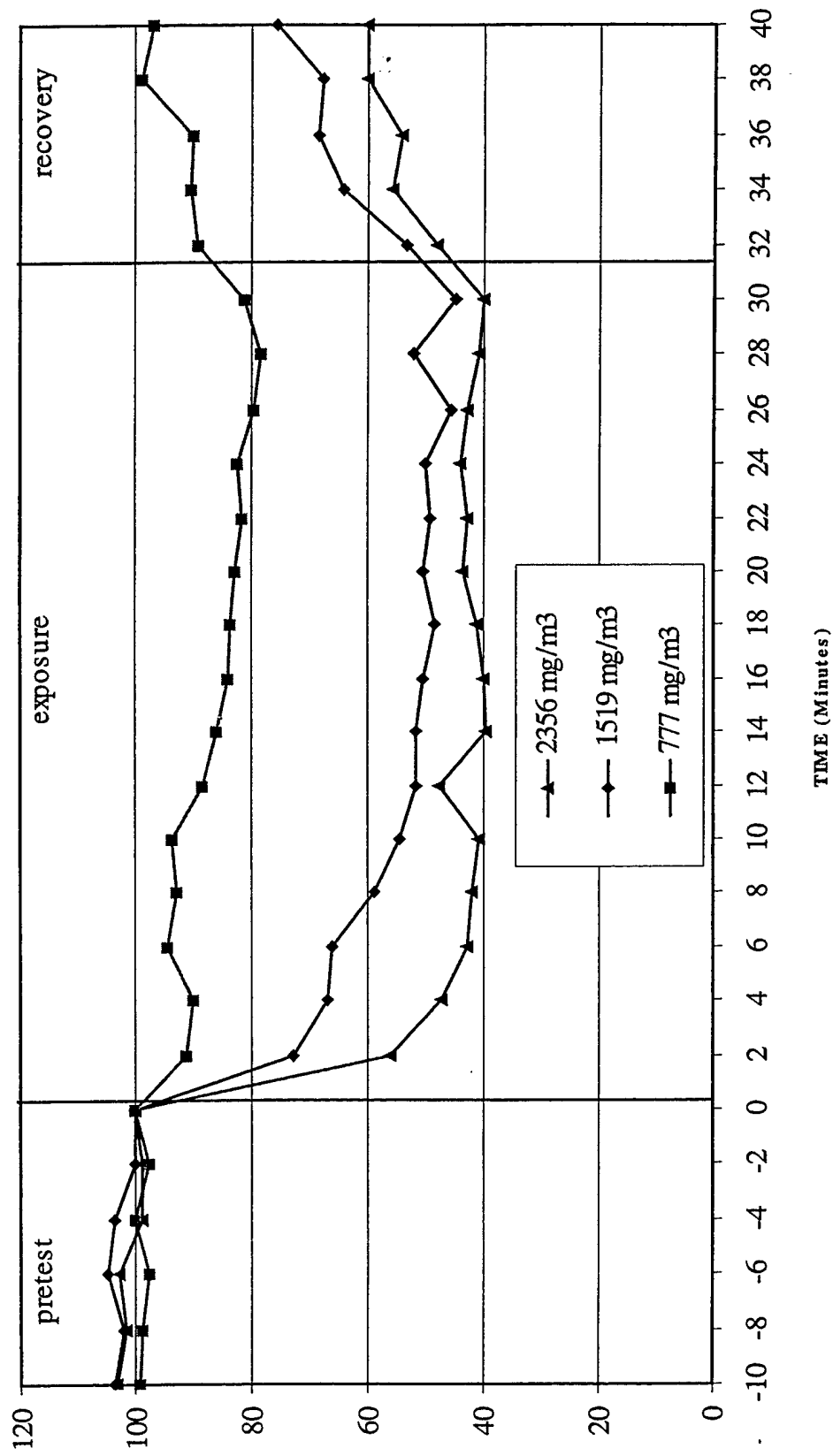


FIGURE 6 - INDIVIDUAL RESPIRATORY RATES: JP-4 (MRD-00-629)

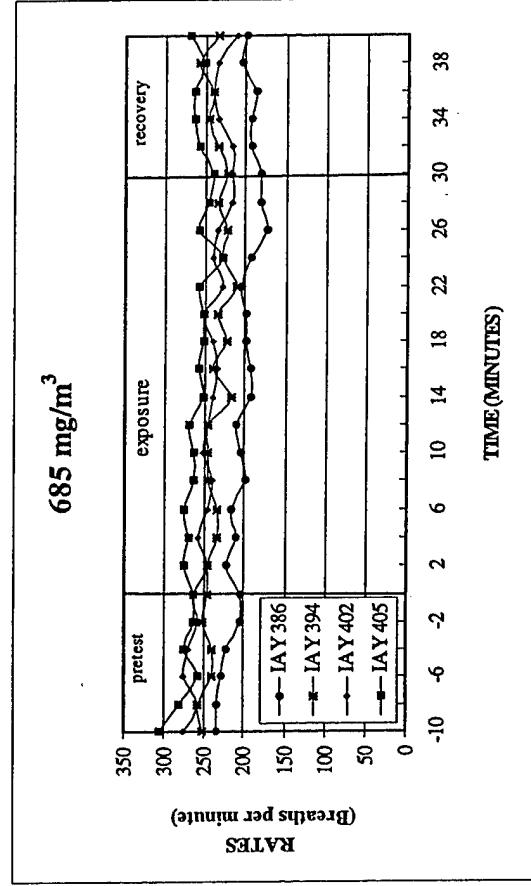
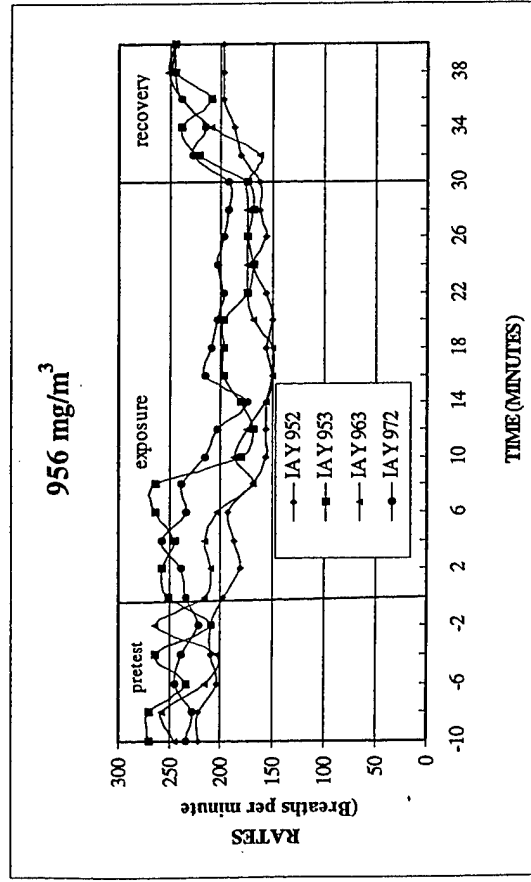
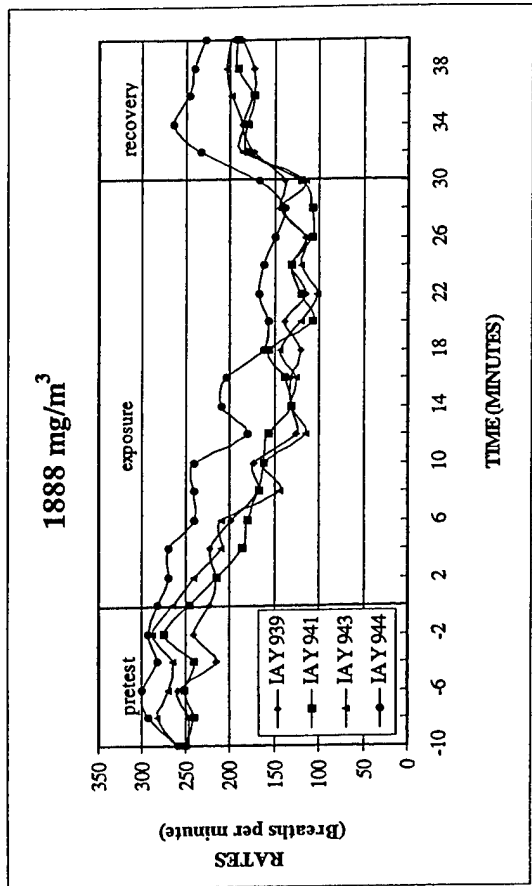
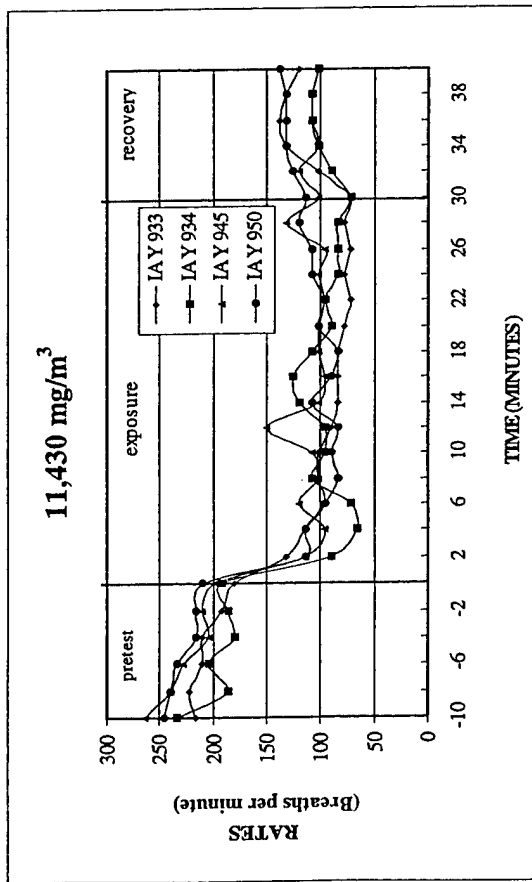


FIGURE 7 - INDIVIDUAL RESPIRATORY RATES: JP-8 (MRD-00-630)

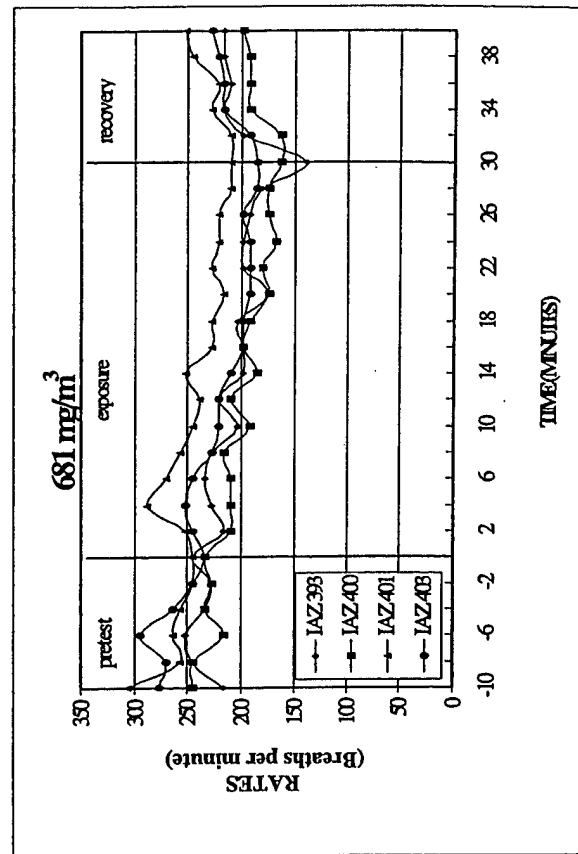
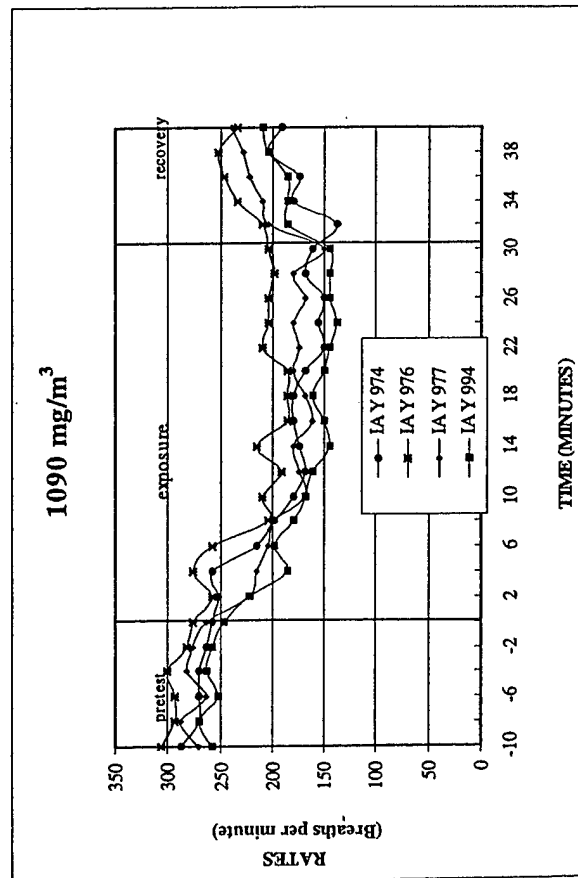
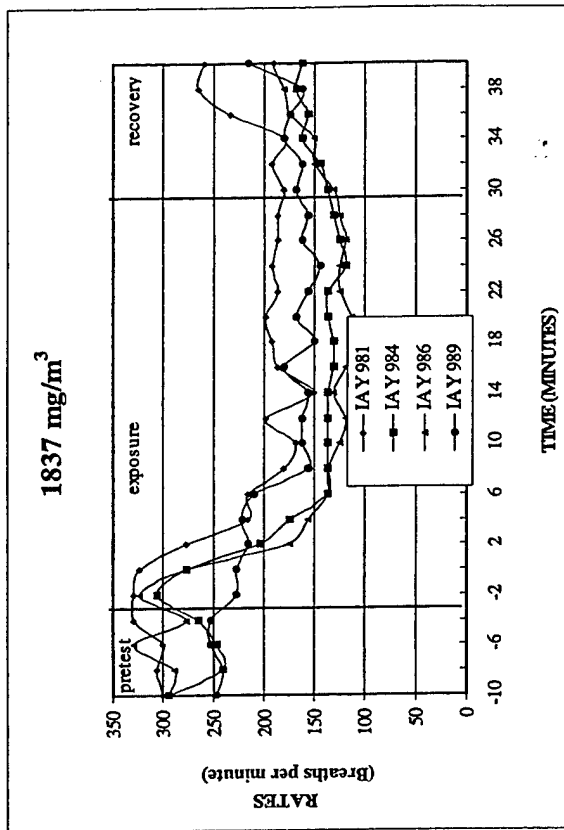
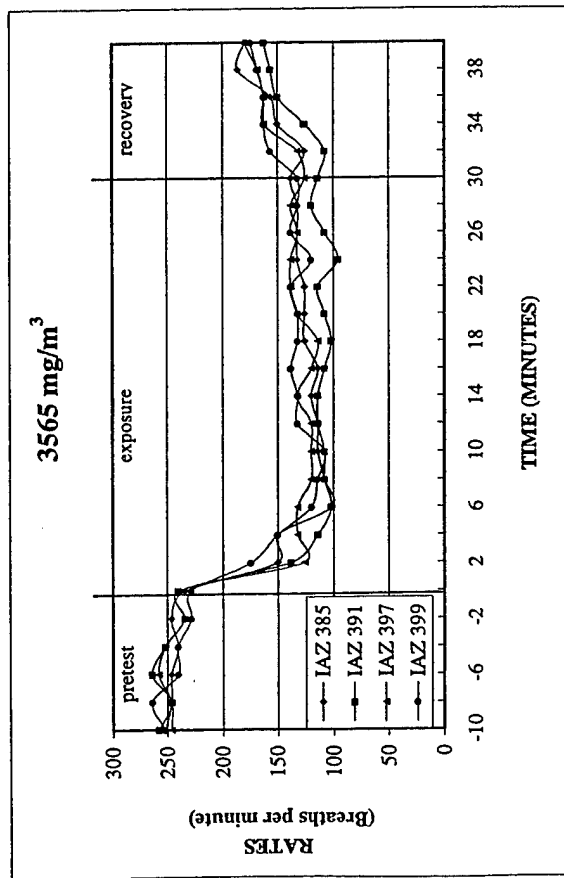


FIGURE 8 - INDIVIDUAL RESPIRATORY RATES: JP-8 (MRD-00-630)
Vapor Only Exposure

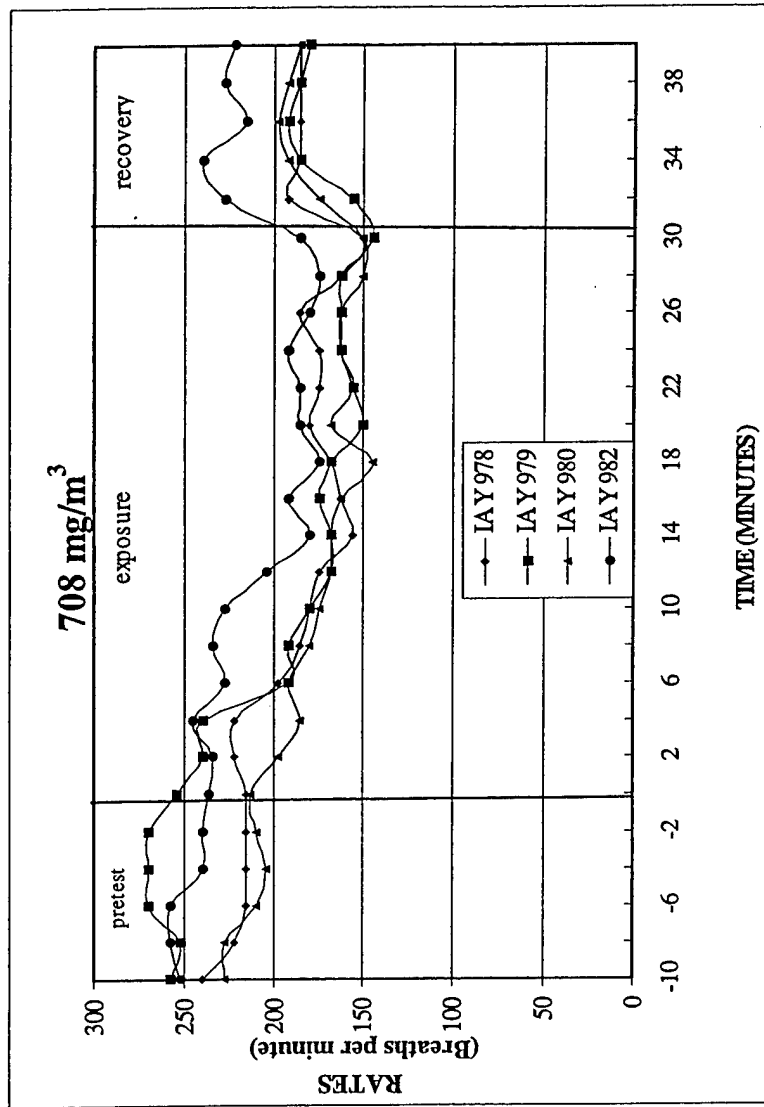


FIGURE 9 - INDIVIDUAL RESPIRATORY RATES: JP-8+100

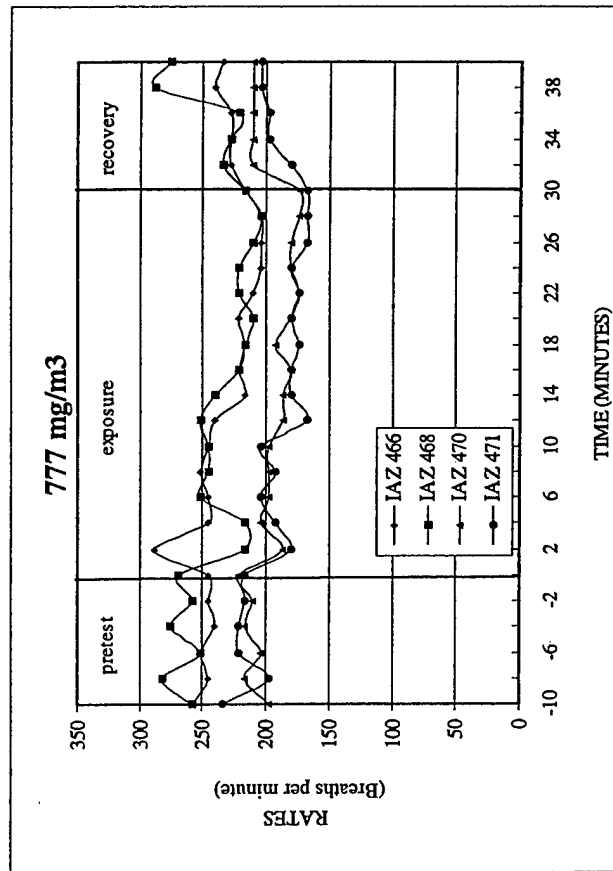
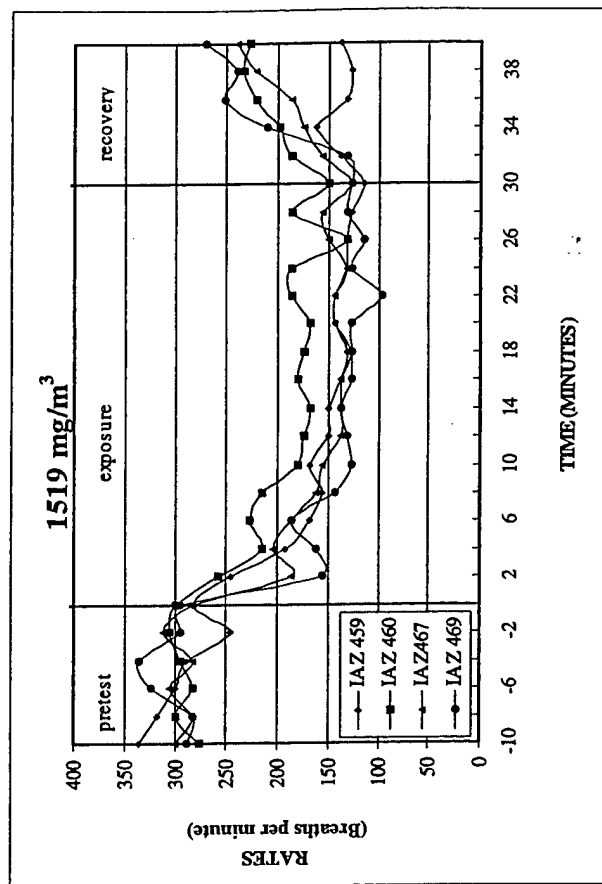
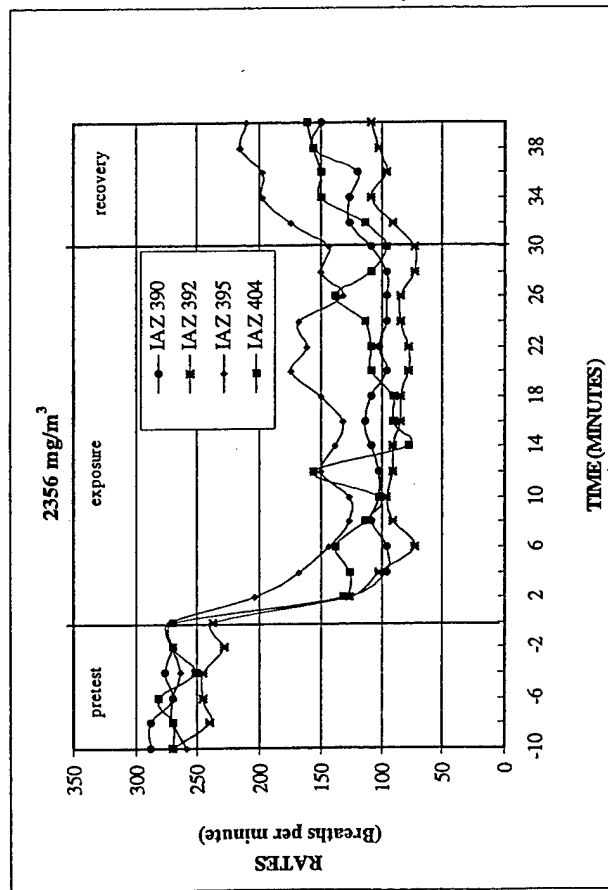


FIGURE 10 - RD₅₀ CALCULATION: JP-4, JP-8, JP-8+100

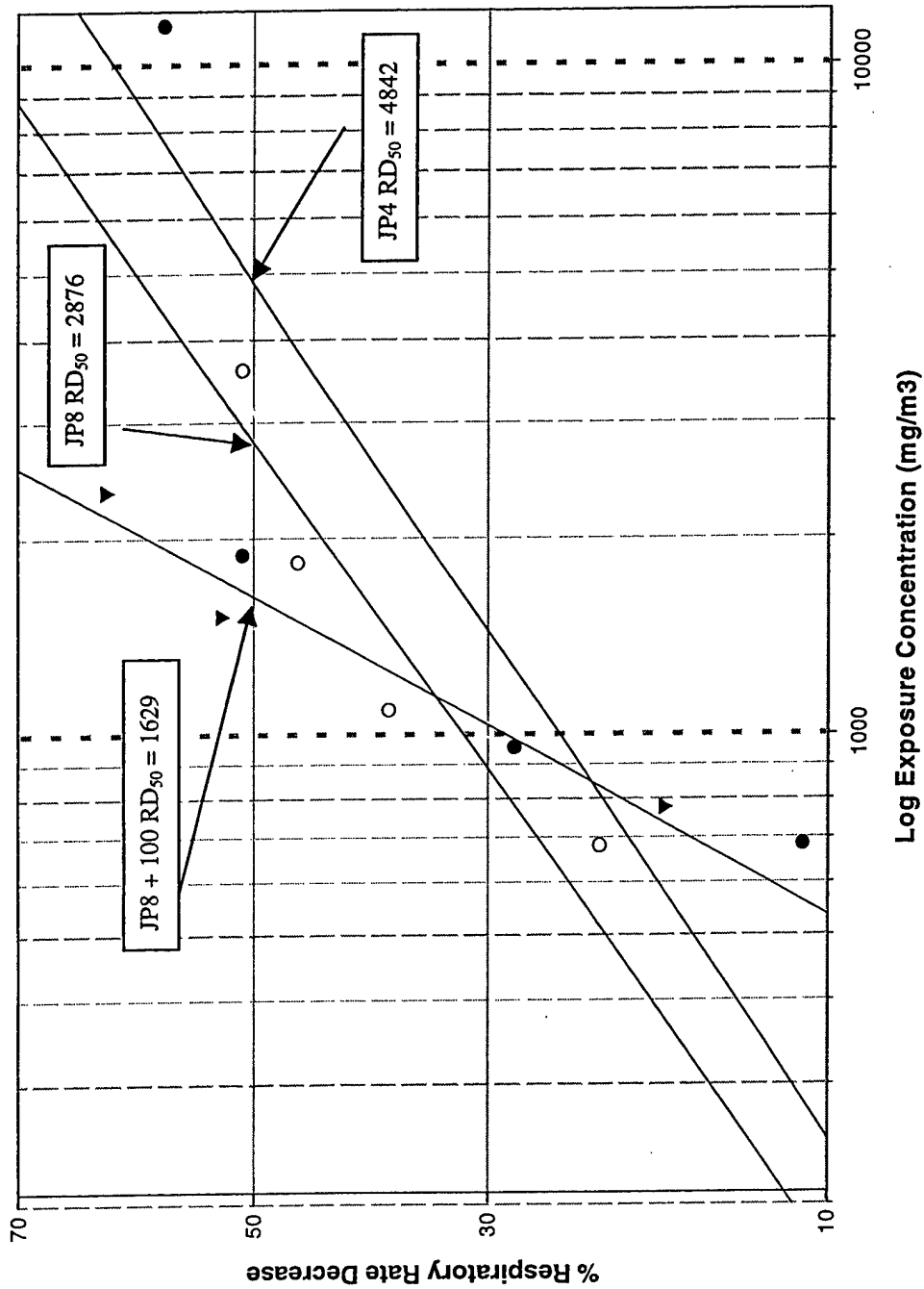


TABLE 5 - SUMMARY OF ANALYTICAL DATA: JP-4 (MRD-00-629)

Sample #:	1	2	3	1	2	3	1	2	3	1	2	3
Non-Volatile Aerosol (filter), mg/m ³	118	82	43	0	0	0	0	0	0	0	0	0
Volatile Hydrocarbons (sorbert tube), mg/m ³	11337	11273	11436	1943	1886	1834	948	1018	901	621	713	722
Total Analytical Concentration, mg/m ³	11455	11355	11479	1943	1886	1834	948	1018	901	621	713	722
Mean Analytical Concentration, mg/m ³	11430 ± 66			1888 ± 55			956 ± 59			685 ± 56		
Nominal Concentration, mg/m ³	33889			4000			1333			1111		
Median Particle Size (um)*	5.35**			* Mass Median equivalent aerodynamic size (50% size). ** insufficient aerosol in sample for valid particle size analysis								
Geometric Standard Deviation (um)	2.21											
% less than or equal to 15 um	90.3											
% less than or equal to 10 um	78.4											
% less than or equal to 1 um	1.7											

TABLE 6 - SUMMARY OF ANALYTICAL DATA: JP-8 (MRD-00-630)

Sample #:	1	2	3	1	2	3	1	2	3	1	2	3
Non-Volatile Aerosol (Filter), mg/m ³	1152	1244	1294	442	465	469	160	166	183	21	25	22
Volatile Hydrocarbons (sorbent tube), mg/m ³	2265	2386	2356	1350	1451	1335	908	945	908	622	698	654
Total Analytical Concentration, mg/m ³	3559	3630	3650	1792	1916	1804	1068	1111	1091	643	723	676
Mean Analytical Concentration, mg/m ³	3613 ± 48			1837 ± 68			1090 ± 22			681 ± 40		
Nominal Concentration, mg/m ³	5333			3333			2000			1111		
Median Particle Size (um)*	* Mass Median equivalent aerodynamic size (50% size).						0.25					
Geometric Standard Deviation (um)							3.89					
% less than or equal to 15 um							99.9					
% less than or equal to 10 um							99.7					
% less than or equal to 1 um							84.7					
							708 ± 33					
										1222		

[illegible]

APPENDIX A : TABLE 1A- ANALYTICAL PROCEDURES AND DETAILED HYDROCARBON ANALYSES

METHOD:

SUBSTANCE(S)
DETECTION LIMIT:
MIN.DET.CONC.:
IHM CLASS:

Hydrocarbons in Air
(See Chem/Phys Properties)
1ug
0.1 ppm
A/B

SAMPLING CONDITIONS:

SAMPLING DEVICE(S):
MANUFACTURER:
CATALOG NUMBER:
SAMPLE STABILITY (days):
SHIPMENT/STORAGE:

Charcoal Tubes (100/50mg, 400/200mg)
SKC, Inc.
226-01, 226-09
>28
Dry Ice/Freezer

ANALYTICAL CONDITIONS:

DESORPTION SOLVENT:
VOLUME (mLs)

Carbon Disulfide (CS₂)
1 (small tube)
2 (large tube)
TIME (min): 30 min
Ambient

DESORPTION CONDITIONS:
TEMP:

SPECIAL INSTRUCTIONS:
INSTRUMENT:

None

COLUMN:

HF5890 (or equivalent)
30M x 0.53 mm id CP-Sil 5 CB
35C (4 min) --> 200 C @ 5C/min

TEMPERATURE PROGRAM:

INJECTOR TEMP (C):

SAMPLE SIZE (uL)

DETECTOR

Flame Ionization (FID)

FLOWS:

FUEL - Hydrogen (H₂) 30 (cc/min)
OXIDANT - Air 375 (cc/min)
CARRIER - Helium (He) or Nitrogen (N₂) 5-10 (cc/min)

TABLE 1A- ANALYTICAL PROCEDURES AND DETAILED HYDROCARBON ANALYSES (CONT'D)

CHEMICAL/PHYSICAL PROPERTIES:

SUBSTANCE (& synonym)	DENSITY (gms/mL)	MOL. WT.	RETENTION TIME (min)
Isopentane	0.620	72.15	2.8
n-Pentane	0.626	72.15	3.3
2-Methylpentane	0.653	86.17	5.4
3-Methylpentane	0.664	86.17	5.9
n-Hexane	0.660	86.17	6.5
Methylcyclopentane	0.749	84.16	7.6
Benzene	0.880	78.11	8.6
Cyclohexane	0.778	84.16	8.9
3-Methylhexane	0.687	100.21	9.6
Iso-Octane	0.692	114.22	10.2
Heptane	0.684	100.20	10.6
Toluene	0.868	92.14	12.9
3-Methylheptane	0.706	114.22	13.7
n-Octane	0.703	114.22	14.7
Ethylbenzene	0.866	106.20	16.7
p&m-Xylene	0.863	106.20	17.0
o-Xylene	0.880	106.20	17.9
Nonane	0.722	106.20	18.5
Cumene	0.862	120.19	19.1
Propylbenzene	0.862	120.19	20.1
p&m-Ethyltoluene	0.863	120.19	20.5
1,3,5-Trimethylbenzene	0.876	120.19	20.7
o-Ethyltoluene	0.881	120.19	21.0
1,2,4-Trimethylbenzene	0.865	120.19	21.6
Decane	0.730	142.30	22.1
n-Undecane	0.740	156.31	25.3
Dodecane	0.749	170.34	28.4
Tetradecane	0.763	198.40	33.8
Hexadecane	0.773	226.45	38.7
Total Hydrocarbons as n-Hexane Equivalents (sum of all peaks minus the solvent peak)			1-40

STOCK STANDARD: 40µL each component / 100mLs CS2

WORKING STANDARDS: 10/25 mL Serial Dilutions of Stock to cover concentration range of interest.

TABLE 2A - INDIVIDUAL HYDROCARBON DATA: JP-4 (MRD-00-629)

(all values are mg/m³)

Group Designation	I			II			III			IV		
	Sorbent Tube	Filter		Sorbent Tube	Filter		Sorbent Tube	Filter		Sorbent Tube	Filter	
Sample Media	11273	82		1886	0		945	0		713	0	
Sample Concentration	3630			1886			1018			713		
Individual Hydrocarbons												
Isopentane	Vapor 36	Aerosol *		Vapor 5	Aerosol *		Vapor 2	Aerosol *		Vapor 2	Aerosol *	
n-Pentane	81	*		11	*		5	*		4	*	
2-Methylpentane	167	*		23	*		11	*		8	*	
3-Methylpentane	92	*		13	*		6	*		4	*	
n-Hexane	347	*		48	*		23	*		15	*	
Methylcyclopentane	307	*		42	*		20	*		14	*	
Benzene	76	*		10	*		5	*		3	*	
Cyclohexane	389	*		54	*		27	*		17	*	
3-Methylhexane	321	*		45	*		22	*		15	*	
Isooctane	415	*		55	*		37	*		25	*	
n-Heptane	720	*		100	*		50	*		33	*	
Toluene	373	*		50	*		35	*		18	*	
3-Methylheptane	198	*		27	*		14	*		10	*	
n-Octane	761	*		109	*		55	*		37	*	
Ethylbenzene	112	*		16	*		9	*		6	*	
p-m-Xylene	NA	n/a		n/a	n/a		n/a	n/a		n/a	n/a	
o-Xylene	NA	n/a		n/a	n/a		n/a	n/a		n/a	n/a	
Xylenes	455	*		64	*		35	*		25	*	
n-Nonane	263	*		41	*		21	*		15	*	
Cumene	23	*		4	*		2	*		2	*	
Propylbenzene	31	*		5	*		3	*		2	*	
p-m Ethyltoluene	74	*		12	*		6	*		5	*	
1,3,5-Trimethylbenzene	112	*		19	*		10	*		7	*	
o-Ethyltoluene	37	*		7	*		3	*		3	*	
1,2,4-Trimethylbenzene	112	*		19	*		10	*		7	*	
n-Decane	169	1		33	*		17	*		11	*	
n-Undecane	143	4		43	*		23	*		16	*	
n-Dodecane	61	9		38	*		22	*		15	*	
n-Tetradecane	2	7		7	*		11	*		7	*	
n-Hexadecane	*	1		*	*		*	*		<1	*	

* below the analytical limit of detection (0.1-0.2 mg/m³ based on sample volume) n/a - Not applicable (Not all samples analyzed for individual xylenes)

TABLE 3A - INDIVIDUAL HYDROCARBON DATA: JP-8 (MRD-00-630)
(all values are mg/m³)

Group Designation		I			II			III			IV			V			
Sample Media	Sorbent Tube	Filter	Sorbent Tube	Filter	Sorbent Tube	Filter	Sorbent Tube	Filter	Sorbent Tube	Filter	Sorbent Tube	Filter	Sorbent Tube	Filter	Sorbent Tube	Filter	
Sample Concentration	2386	1244	3613		1451	465	1018	166	1111		698	25	713		713	*	
Total Concentration	3613				1916				1111				723				713
Individual Hydrocarbons	Vapor	Aerosol		Vapor	Aerosol		Vapor	Aerosol		Vapor	Aerosol		Vapor	Aerosol		Aerosol	
Isopentane	*	*		*	*		2	*		*	*		*	*		*	
n-Pentane	*	*		*	*		5	*		*	*		*	*		*	
2-Methylpentane	1	*		*	*		11	*		*	*		*	*		*	
3-Methylpentane	1	*		*	*		6	*		*	*		*	*		*	
n-Hexane	1	*		*	*		23	*		*	*		*	*		*	
Methylcyclopentane	2	*		*	*		20	*		*	*		*	*		*	
Benzene	1	*		*	*		5	*		*	*		*	*		*	
Cyclohexane	2	*		*	*		27	*		*	*		*	*		*	
3-Methylhexane	4	*		*	*		22	*		*	*		*	*		*	
Isooctane	6	*		1	*		37	*		*	2		*	*		*	
n-Heptane	9	*		2	*		50	*		*	3		1	*		*	
Toluene	12	*		4	*		25	*		*	3		1	*		*	
3-Methylheptane	10	*		4	*		14	*		*	2		1	*		*	
n-Octane	35	*		18	*		55	*		*	9		3	*		*	
Ethylbenzene	16	*		9	*		9	*		*	4		2	*		*	
p-m-Xylene	n/a	n/a		16	n/a		n/a	n/a		n/a	n/a		n/a	n/a		n/a	
o-Xylene	n/a	n/a		7	n/a		n/a	n/a		n/a	n/a		n/a	n/a		n/a	
Xylenes	52	*		23	*		35	*		*	11		4	*		*	
n-Nonane	71	2		39	*		21	*		*	17		10	*		*	
Cumene	14	*		8	*		2	*		*	3		2	*		*	
Propylbenzene	20	1		11	*		3	*		*	5		3	*		*	
p-m Ethyltoluene	45	2		25	*		6	*		*	11		7	*		*	
1,3,5-Trimethylbenzene	80	4		45	1		10	*		*	19		15	*		*	
o-Ethyltoluene	37	2		21	*		3	*		*	9		7	*		*	
1,2,4-Trimethylbenzene	71	4		39	1		10	*		*	17		14	*		*	
n-Decane	135	13		77	3		17	0		*	32		31	*		*	
n-Undecane	171	47		107	11		23	2		*	43		59	*		*	
n-Dodecane	93	82		69	24		22	6		*	32		51	*		*	
n-Tetradecane	9	87		11	5		11	17		*	12		15	*		0	
n-Hexadecane	*	23		*	11		*	6		*	2		2	*		0	

* below the analytical limit of detection (0.1-0.2 mg/m³ based on sample volume) n/a - Not applicable (Not all samples analyzed for individual xylenes)

TABLE 4A - INDIVIDUAL HYDROCARBON DATA: JP-8+100 (MRD-00-631)
(all values are mg/m³)

Group/Designation		I			II			III		
Sample Media	Sorbent Tube	Filter	Sorbent Tube	Filter	Sorbent Tube	Filter	Sorbent Tube	Filter		
Sample Concentration	1771	615	1220	297	725	31				
Total Concentration	2386		1517		777					
Individual Hydrocarbons:		Vapor	Aerosol	Vapor	Aerosol	Vapor	Aerosol			
Isopentane	*	1	*	*	2	*				
n-Pentane	*	*	*	*	2	*				
2-Methylpentane	2	*	1	*	3	*				
3-Methylpentane	1	*	*	*	2	*				
n-Hexane	3	*	1	*	3	*				
Methylcyclopentane	3	*	1	*	3	*				
Benzene	1	*	1	*	1	*				
Cyclohexane	3	*	1	*	3	*				
3-Methylhexane	6	*	2	*	4	*				
Isooctane	9	*	4	*	6	*				
n-Heptane	14	*	6	*	9	*				
Toluene	17	*	8	*	9	*				
3-Methylheptane	11	*	7	*	6	*				
n-Octane	38	*	23	*	18	*				
Ethylbenzene	16	*	10	*	7	*				
p-m-Xylene	44	*	n/a	n/a	n/a	n/a				
o-Xylene	12	*	n/a	n/a	n/a	n/a				
Xylenes	56	*	37	23	24	*				
n-Nonane	57	*	39	*	23	*				
Cumene	11	*	7	*	4	*				
Propylbenzene	15	*	10	*	5	*				
p-m Ethyltoluene	33	*	23	*	13	*				
1,3,5-Trimethylbenzene	46	*	30	0	16	*				
o-Ethyltoluene	37	*	25	0	14	*				
1,2,4-Trimethylbenzene	47	1	31	62	16	*				
n-Decane	100	4	67	1	34	*				
n-Undecane	129	19	89	4	42	*				
n-Dodecane	77	40	61	10	30	1				
n-Tetradecane	10	57	14	25	11	4				
n-Hexadecane	*	18	6	9	1	2				

* below the analytical limit of detection (0.1-0.2 mg/m3 based on sample volume) n/a - Not applicable (Not all samples analyzed for individual xylenes)

TABLE 5A - INDIVIDUAL HYDROCARBON DATA: PARTICLE SIZE SAMPLES
JP-4 (MRD-00-629)

Test Substance	JP-4				JP-8				JP-8+100			
	11430 mg/m ³				1090 mg/m ³				777 mg/m ³			
	82 mg/m ³				920 mg/m ³				29 mg/m ³			
Total Exposure Concentration	2.60 um	4.10 um	6.80 um	81	0.30 um	0.84 um	1.50 um	0.54 um	1.50 um	0.54 um	1.50 um	2.60 um
Aerosol Concentration	1	32	81		0	78	92	0	63	0	63	88
Stage Constant (cutoff diameter)												
% aerosol less than												
Individual Hydrocarbons:												
Isopentane	*	*	*		*	*	*	*	0	*	0	*
n-Pentane	*	*	*		*	*	*	*	0	*	0	*
2-Methylpentane	*	*	*		*	*	*	*	0	*	0	*
3-Methylpentane	*	*	*		*	*	*	*	0	*	0	*
n-Hexane	*	*	*		*	*	*	*	0	*	0	*
Methylcyclopentane	*	*	*		*	*	*	*	0	*	0	*
Benzene	*	*	*		*	*	*	*	0	*	0	*
Cyclohexane	*	*	*		*	*	*	*	0	*	0	*
3-Methylhexane	*	*	*		*	*	*	*	0	*	0	*
Isooctane	*	*	*		*	*	*	*	0	*	0	*
n-Heptane	*	*	*		*	*	*	*	0	*	0	*
Toluene	*	*	*		*	*	*	*	0	*	0	*
3-Methylheptane	*	*	*		*	*	*	*	0	*	0	*
n-Octane	*	*	*		*	*	*	*	0	*	0	*
Ethylbenzene	*	*	*		*	*	*	*	0	*	0	*
P-M Xylene	n/a	n/a	n/a		n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
O-Xylene	n/a	n/a	n/a		n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Xylenes	*	*	*		*	*	*	*	0	*	0	*
n-Nonane	*	*	*		*	*	*	*	0	*	0	*
Cumene	*	*	*		*	*	*	*	0	*	0	*
Propylbenzene	*	*	*		*	*	*	*	0	*	0	*
p-m Ethyltoluene	*	*	*		*	*	*	*	0	*	0	*
1,3,5-Trimethylbenzene	*	*	*		*	*	*	*	0	*	0	*
o-Ethyltoluene	*	*	*		*	*	*	*	0	*	0	*
1,2,4-Trimethylbenzene	*	*	*		*	*	*	*	0	*	0	*
n-Decane	*	*	*		*	0.087	*	*	0.16	*	0.16	*
n-Undecane	*	*	*		0.067	0.393	0.247	*	0	*	0	*
n-Dodecane	*	*	*		0.147	0.860	0.560	*	0.14	*	0.14	*
n-Tetradecane	*	*	*		0.793	3.20	2.427	*	0.37	*	0.37	*
n-Hexadecane	*	*	*		0.420	1.54	0.827	*	0.28	*	0.28	*

* below the analytical limit of detection (0.1-0.2 mg/m3 based on sample volume) n/a - Not applicable (Not all samples analyzed for individual xylenes)

TABLE 6A - INDIVIDUAL HYDROCARBON DATA - NEAT TEST SUBSTANCES

Individual Hydrocarbons*	JP 4	JP 8	JP 8 + 100
	MRD-00-629	MRD-00-630	MRD-00-631
	Percent By Volume*		
Isopentane	0.1437	ND	ND
n-Pentane	0.3237	ND	ND
2-Methylpentane	0.7302	ND	ND
3-Methylpentane	0.4139	ND	ND
n-Hexane	1.5403	0.0011	0.0023
Methylcyclopentane	1.3913	0.0006	0.0014
Benzene	0.3551	ND	ND
Cyclohexane	1.7803	0.0033	0.0030
3-Methylhexane	1.5065	0.0119	0.0078
Isooctane	2.4941	0.0256	0.0112
n-Heptane	3.3458	0.0481	0.0357
Toluene	2.0009	0.0721	0.0664
3-Methylheptane	0.9567	0.0604	0.0524
n-Octane	3.8056	0.2609	0.2506
Ethylbenzene	0.6458	0.1414	0.1322
P-M Xylene	3.3541	0.6610	0.6319
n-Nonane	1.5714	0.9103	0.8886
Cumene	0.1870	0.1756	0.1672
Propylbenzene	0.1830	0.2846	0.2698
p-m Ethyltoluene	0.5318	0.6921	0.6801
1,3,5-Trimethylbenzene	0.5948	1.0785	1.0677
o-Ethyltoluene	0.4586	0.8522	0.8416
1,2,4-Trimethylbenzene	0.8171	1.2355	1.2192
n-Decane	1.2687	2.8907	2.8641
n-Undecane	1.7350	5.5171	5.5065
n-Dodecane	1.8808	5.3191	5.3032
n-Tetradecane	1.4537	3.0523	3.0658
n-Hexadecane	0.3169	0.7690	0.7602
Total Analytes*	35.7868	24.0634	23.8289

* percent of total test substance analyzed. Total analytes = sum of the 28 individual hydrocarbons; remainder is other unidentified hydrocarbons.
ND - None Detected

FIGURE 1A - CHROMATOGRAM FOR NEAT SUBSTANCE
JP - 4

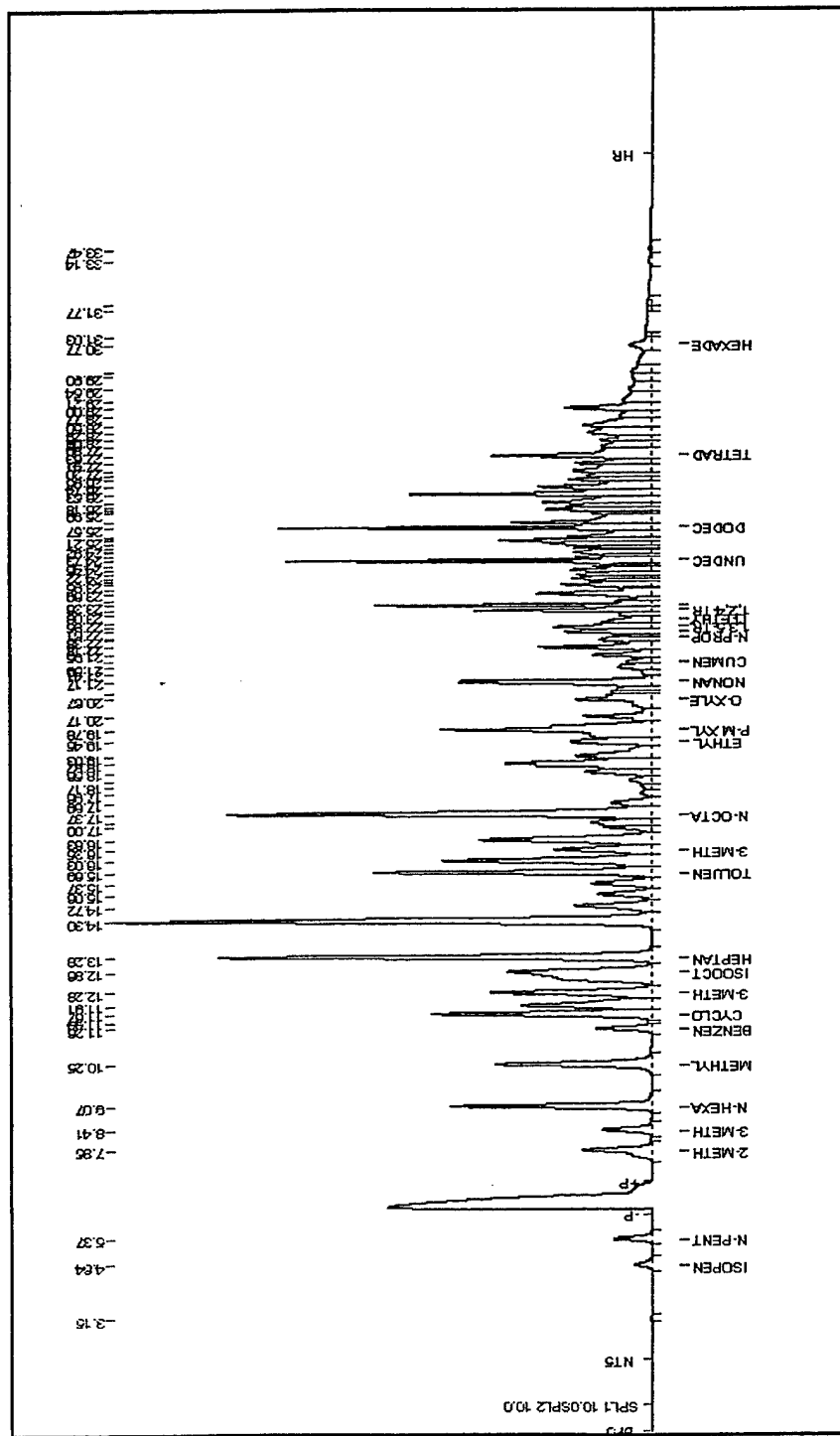


FIGURE 2A CHROMATOGRAM FOR NEAT SUBSTANCE
JP - 8

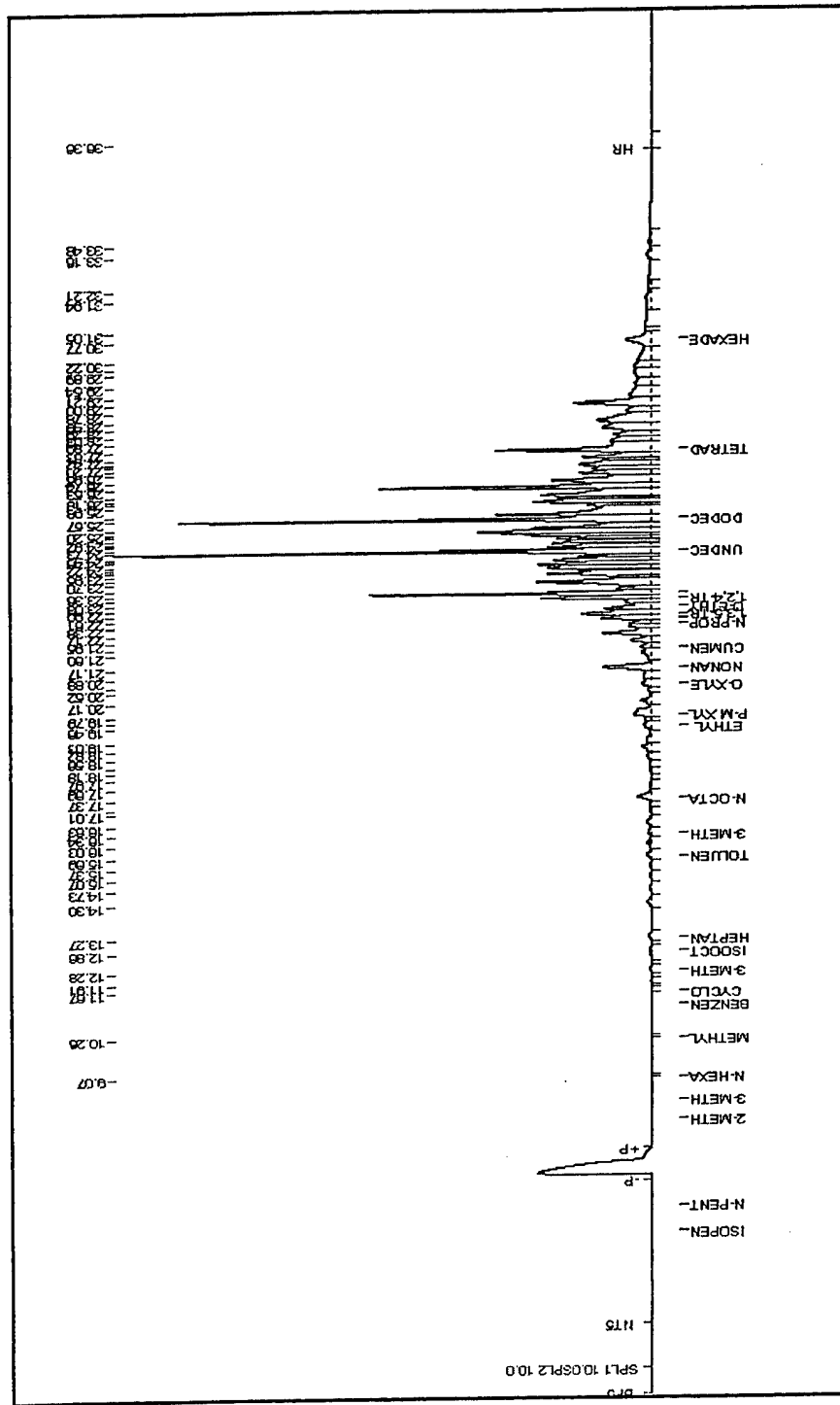


FIGURE 3A - CHROMATOGRAM FOR NEAT SUBSTANCE
JP - 8 + 100

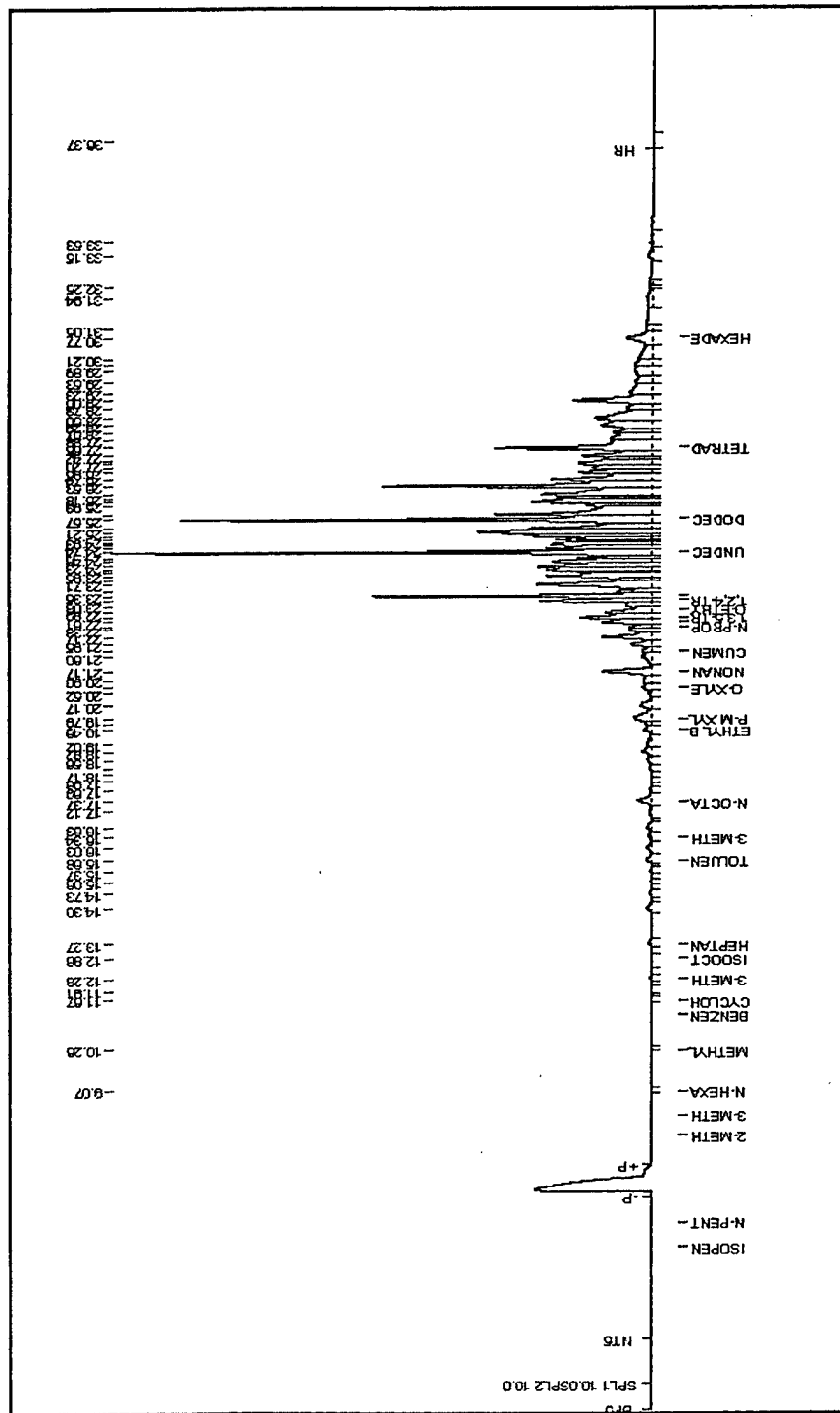


FIGURE 4A - COMPARISON OF CHROMATOGRAMS FOR SELECTED INDIVIDUAL AEROSOL AND VAPOR SAMPLES
JP - 4 (Mean Concentration 11430 mg/m³)

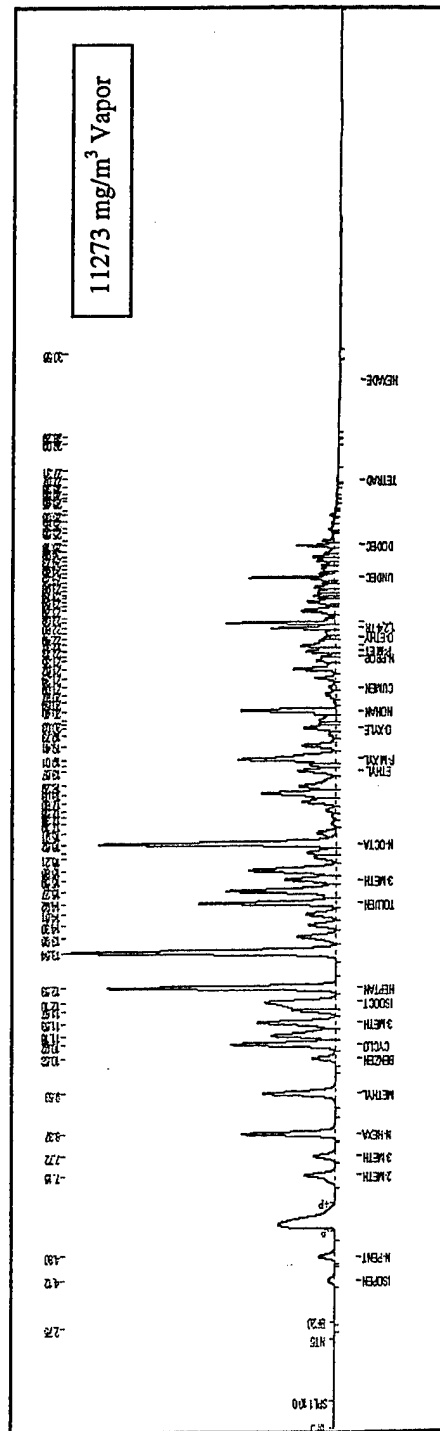
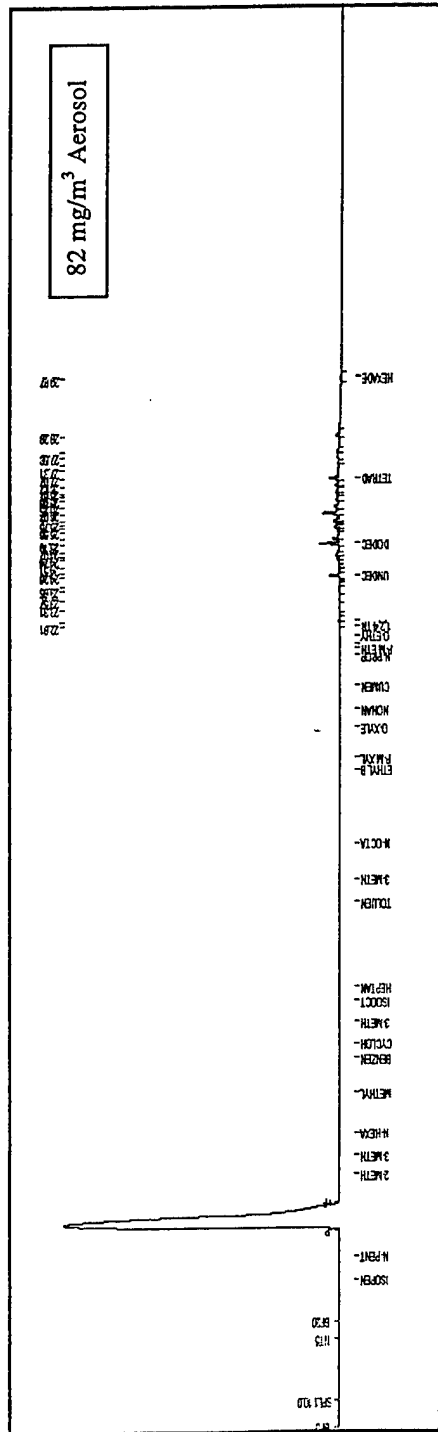


FIGURE 5A - COMPARISON OF CHROMATOGRAMS FOR SELECTED INDIVIDUAL AEROSOL AND VAPOR SAMPLES
JP - 4 (Mean Concentration 1888 mg/m³)

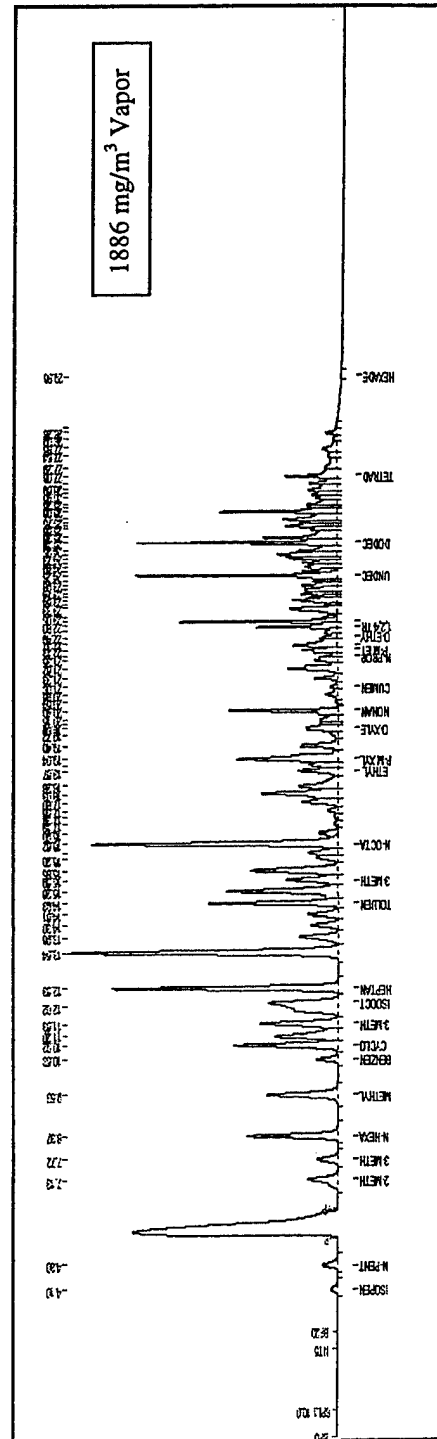
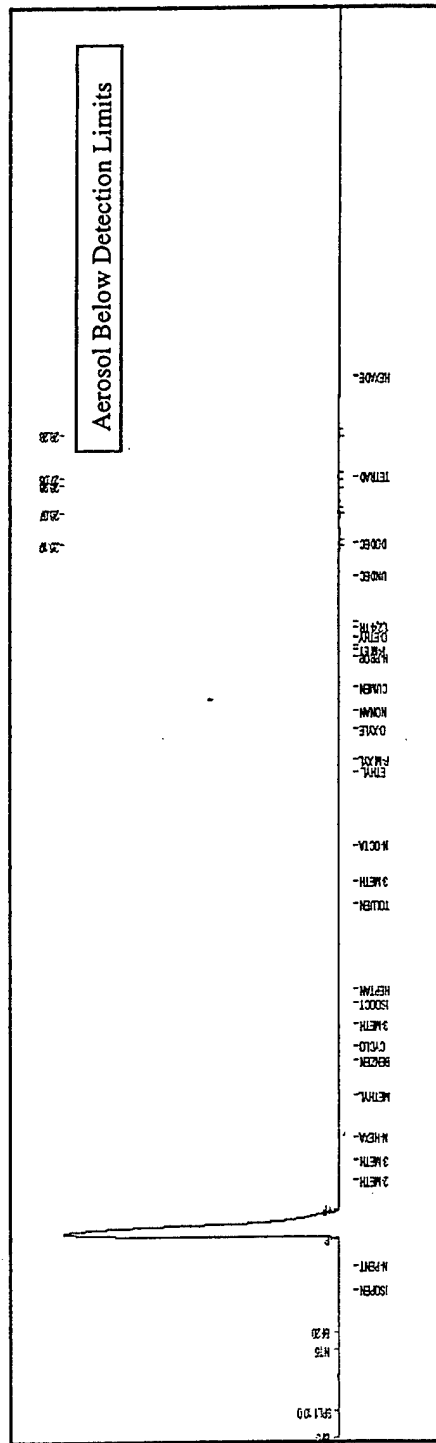
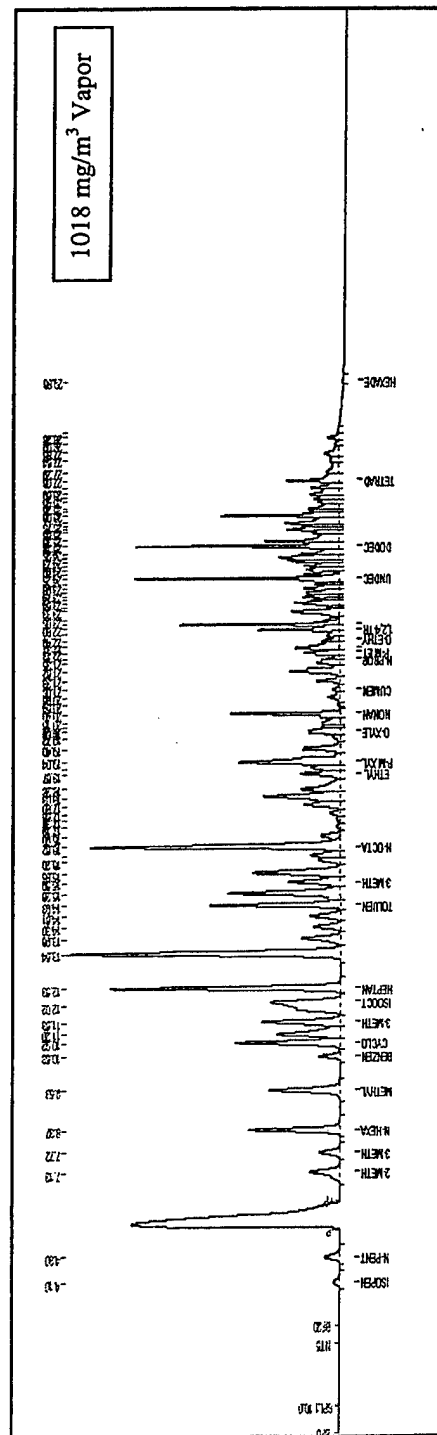
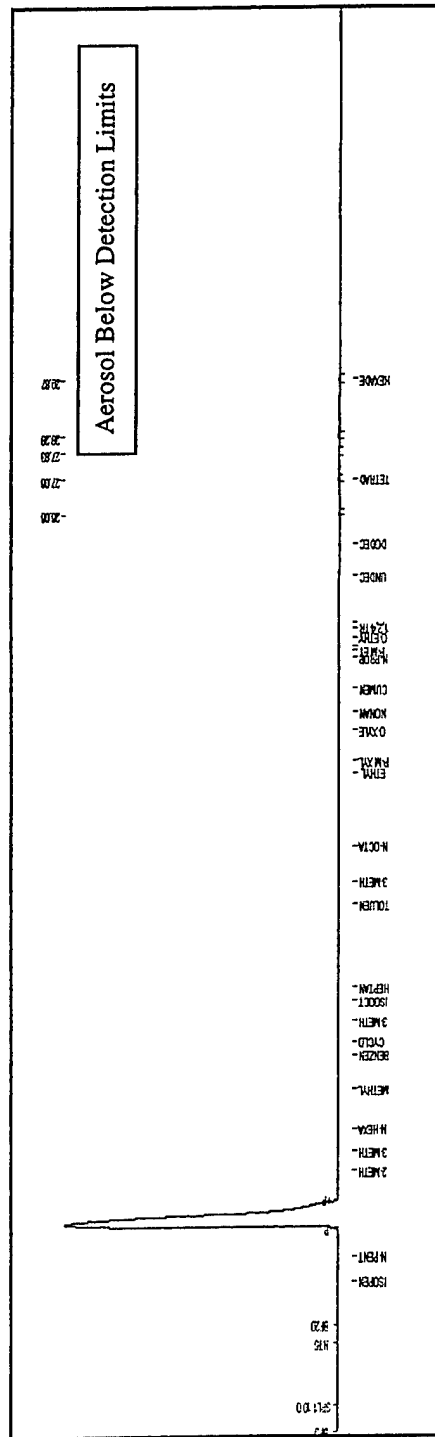


FIGURE 6A - COMPARISON OF CHROMATOGRAMS FOR SELECTED INDIVIDUAL AEROSOL AND VAPOR SAMPLES
JP - 4 (Mean Concentration 956 mg/m³)



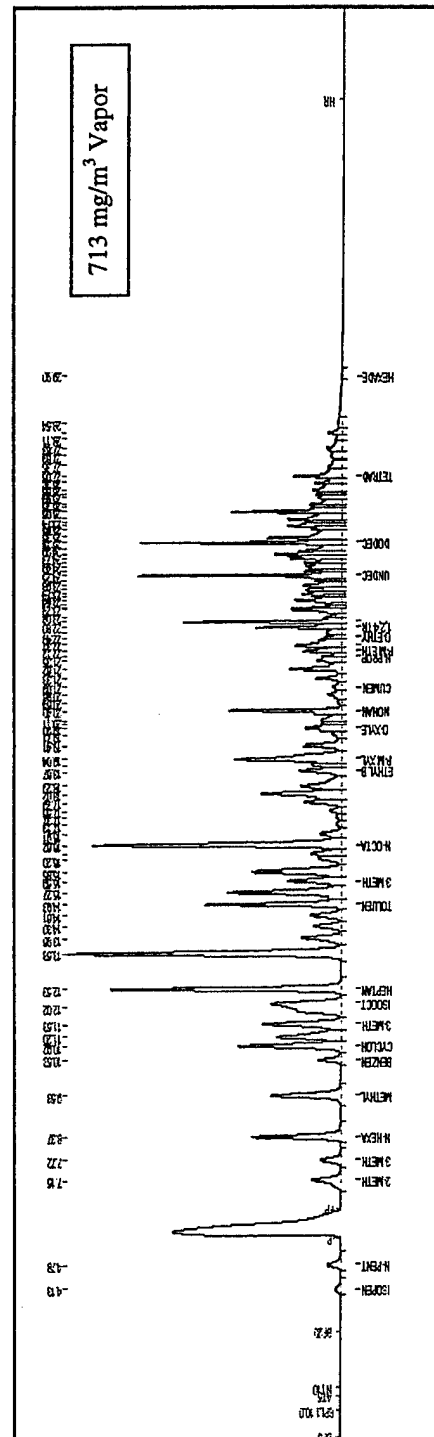
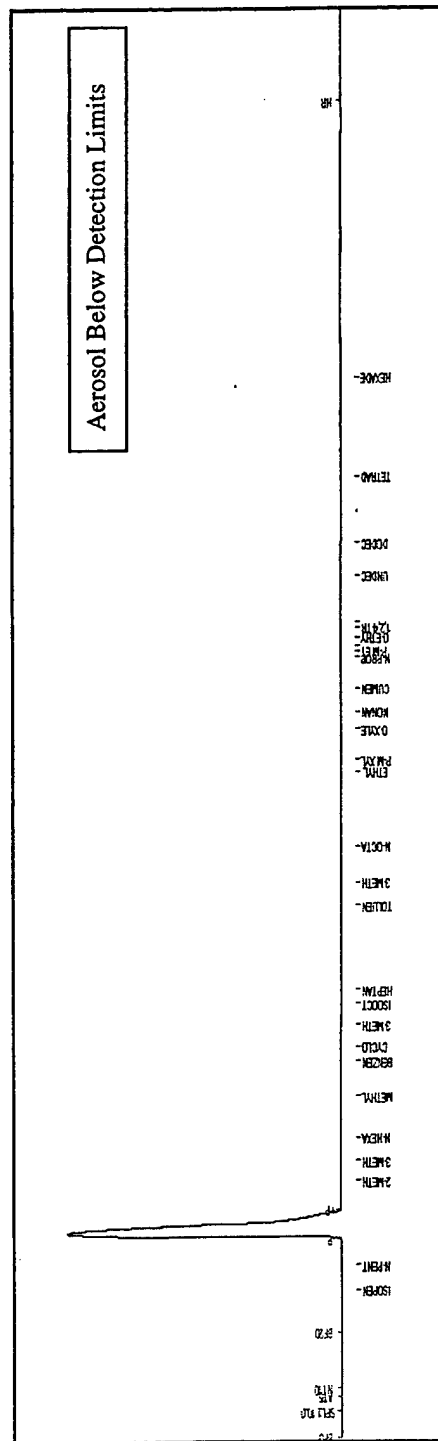


FIGURE 8A - COMPARISON OF CHROMATOGRAMS FOR SELECTED INDIVIDUAL AEROSOL AND VAPOR SAMPLES
JP - 8 (Mean Concentration 3565 mg/m³)

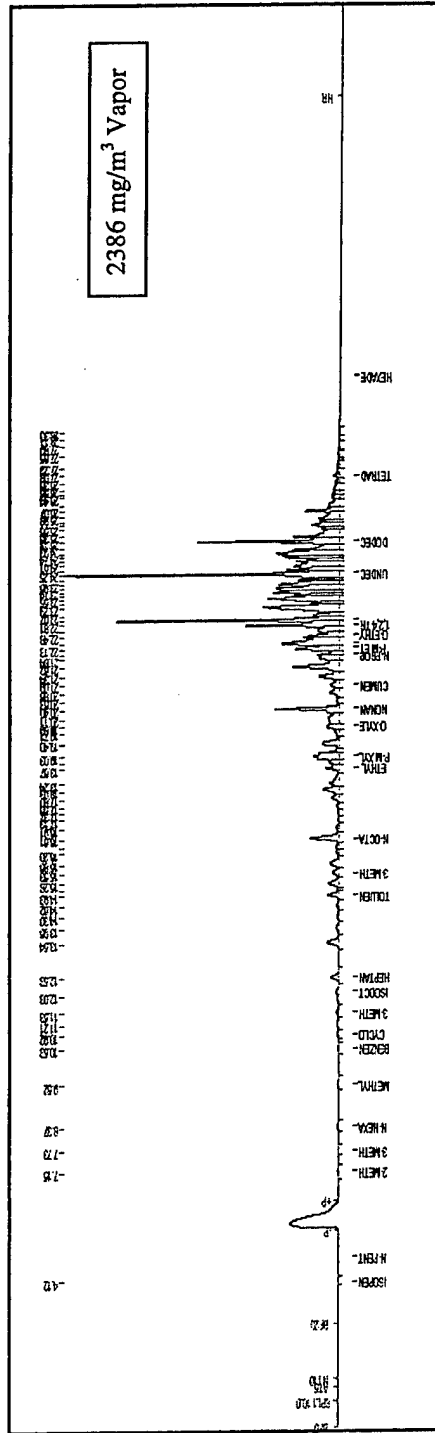
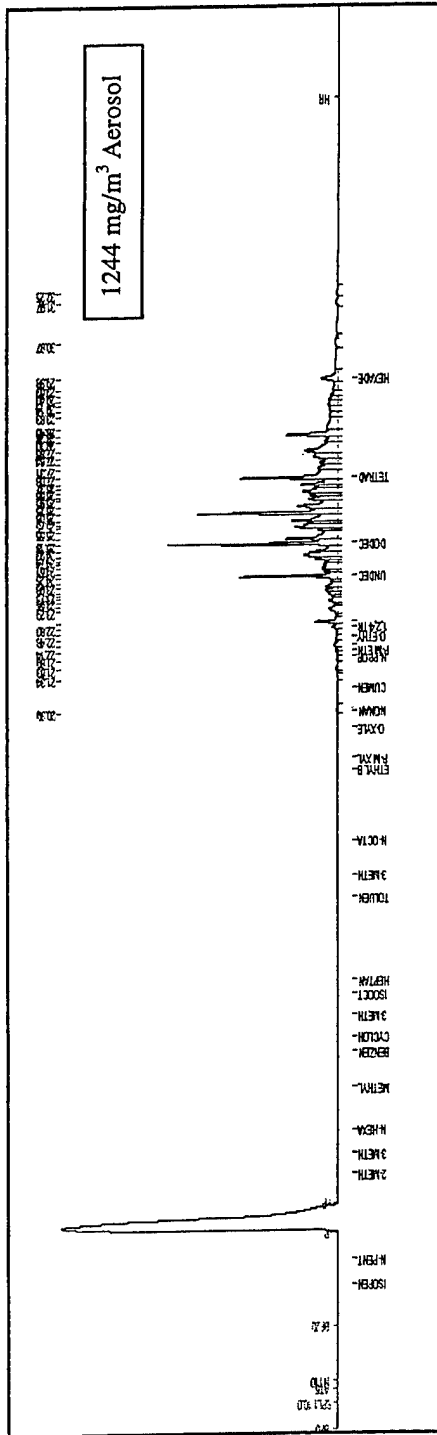


FIGURE 9A - COMPARISON OF CHROMATOGRAMS FOR SELECTED INDIVIDUAL AEROSOL AND VAPOR SAMPLES
JP - 8 (Mean Concentration 1837 mg/m³)

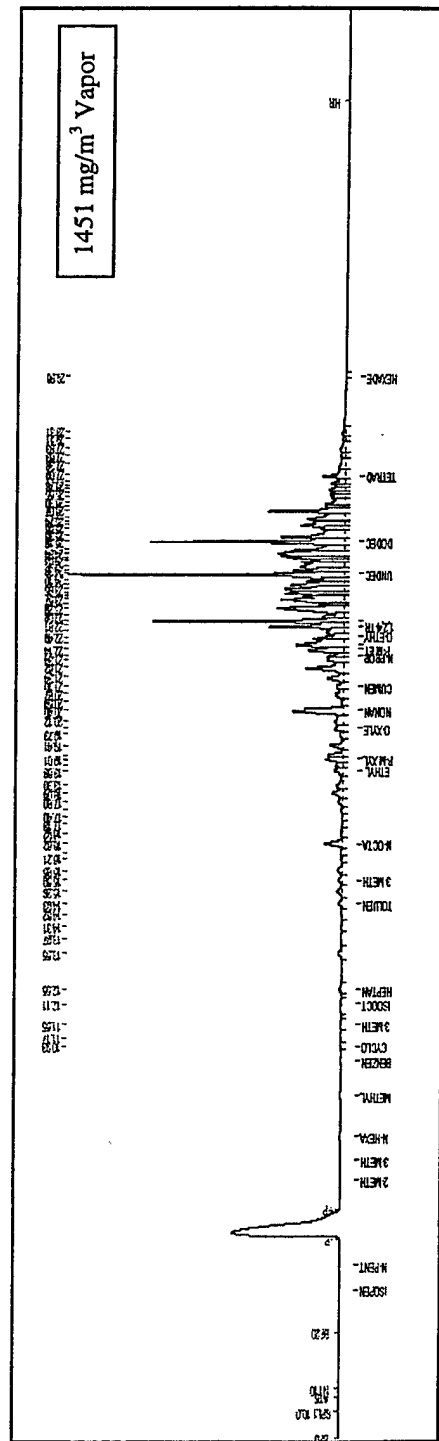
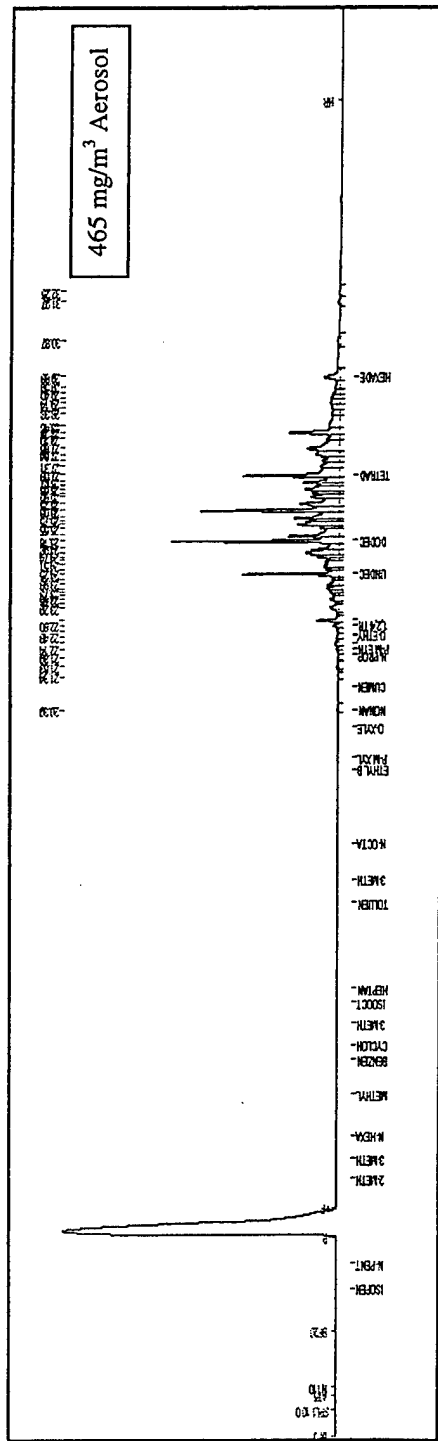


FIGURE 10A - COMPARISON OF CHROMATOGRAMS FOR SELECTED INDIVIDUAL AEROSOL AND VAPOR SAMPLES
JP - 8 (Mean Concentration 1090 mg/m³)

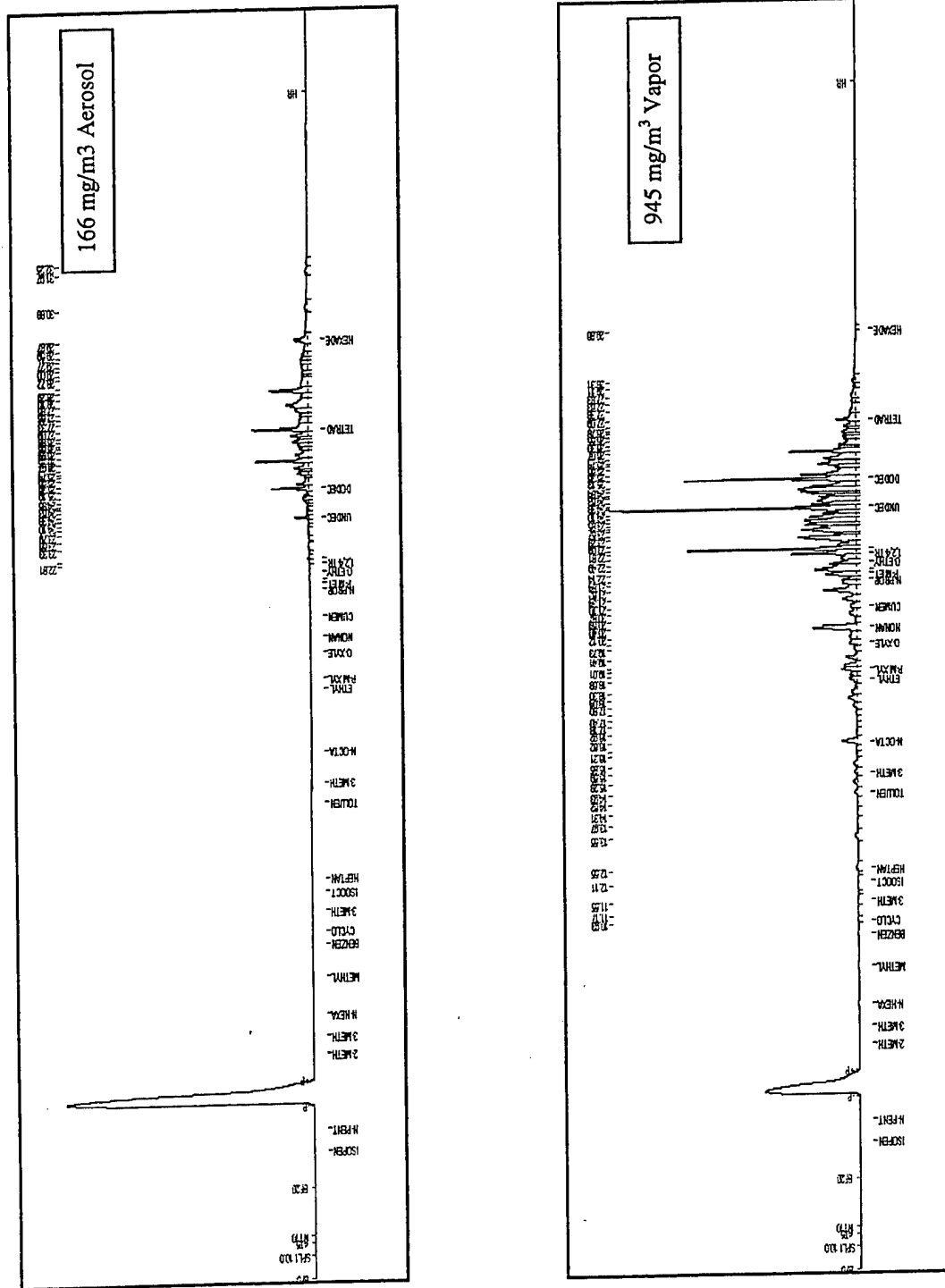
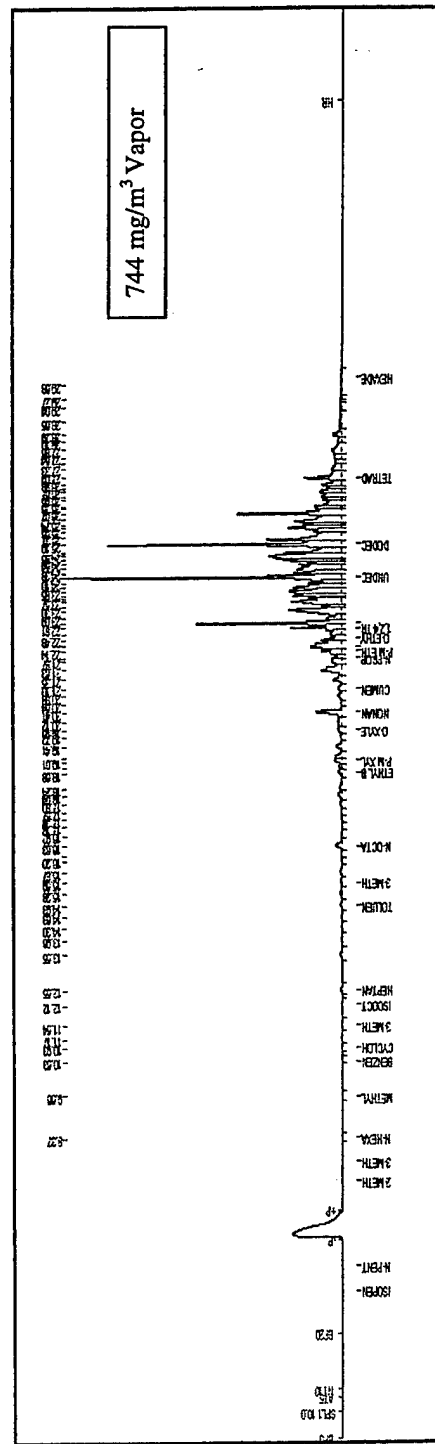
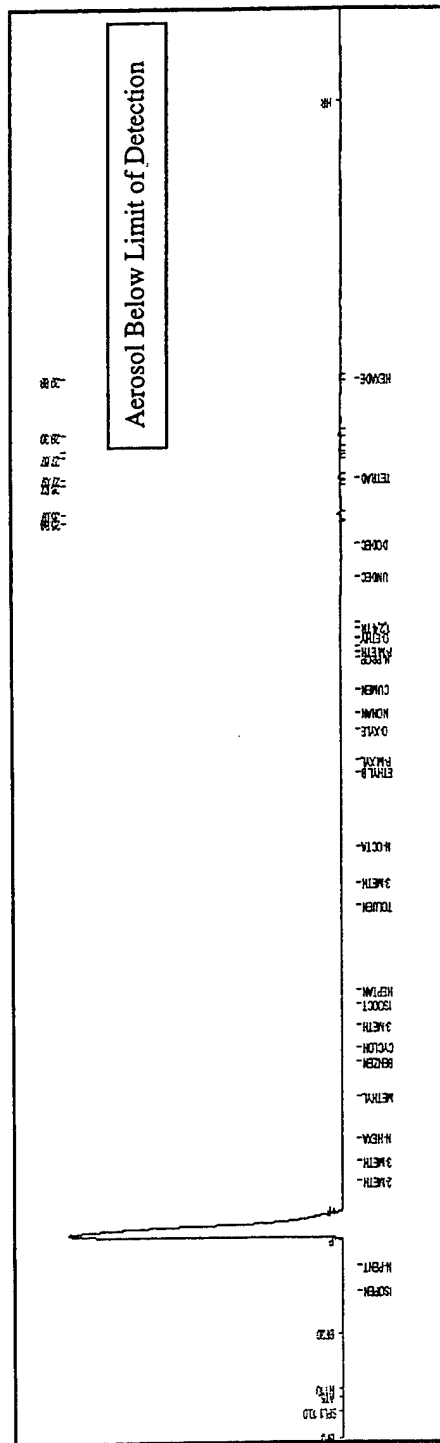
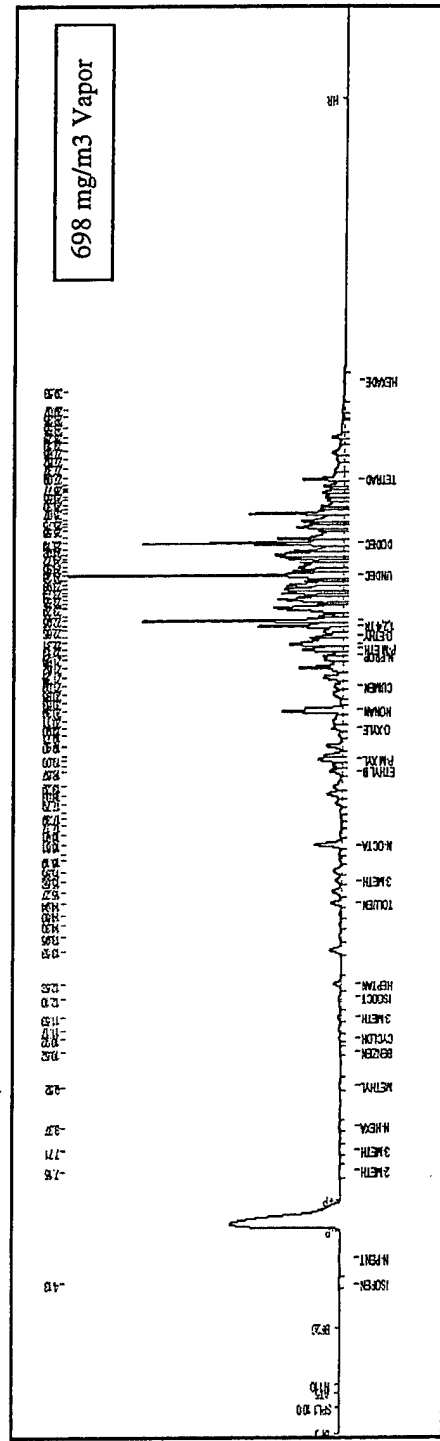
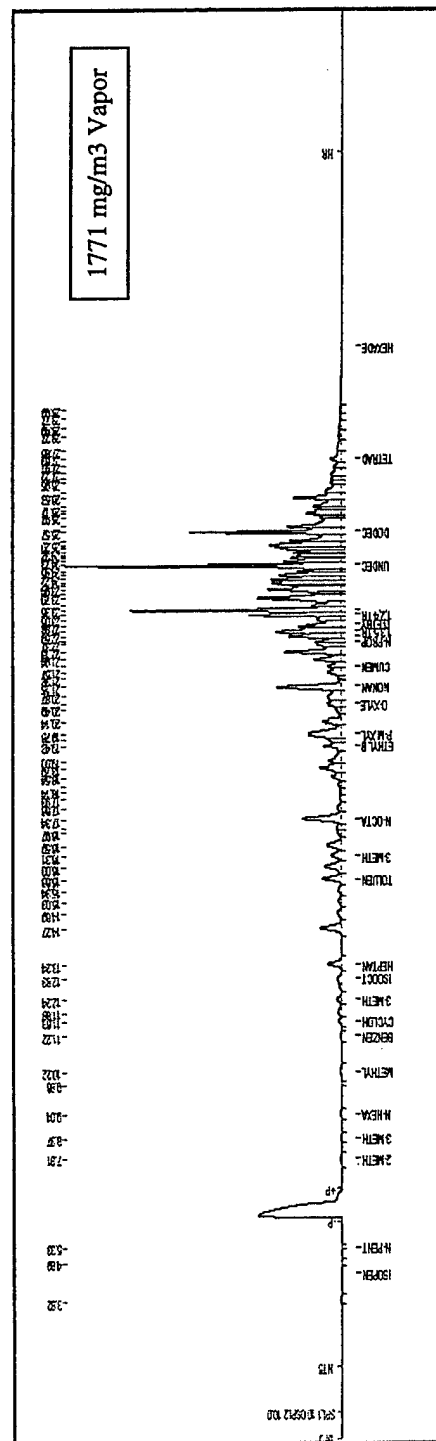
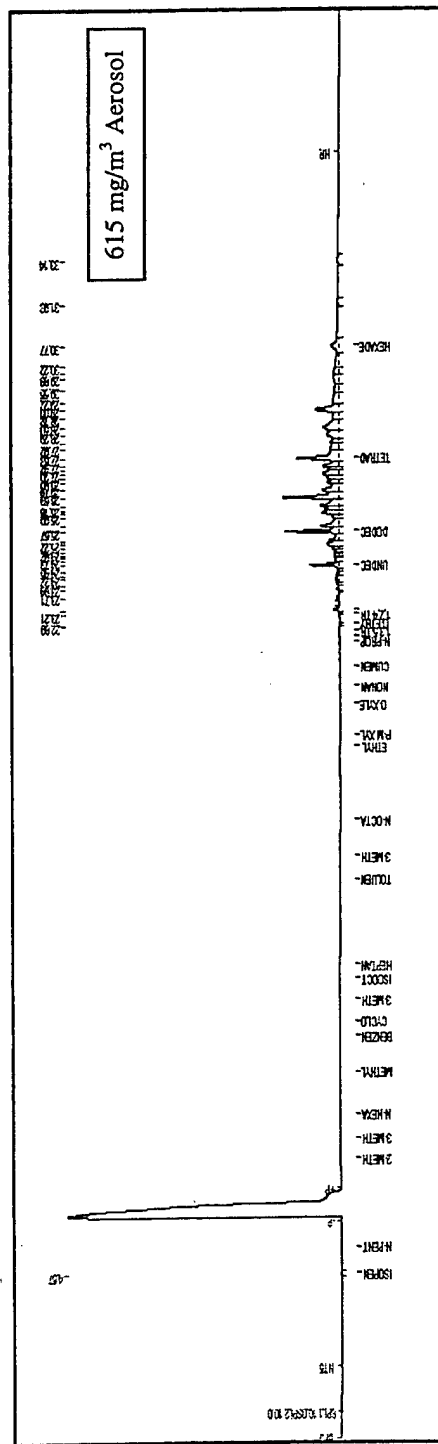


FIGURE 11A - COMPARISON OF CHROMATOGRAMS FOR SELECTED INDIVIDUAL AEROSOL AND VAPOR SAMPLES
JP - 8 (Mean Concentration 681 mg/m³)







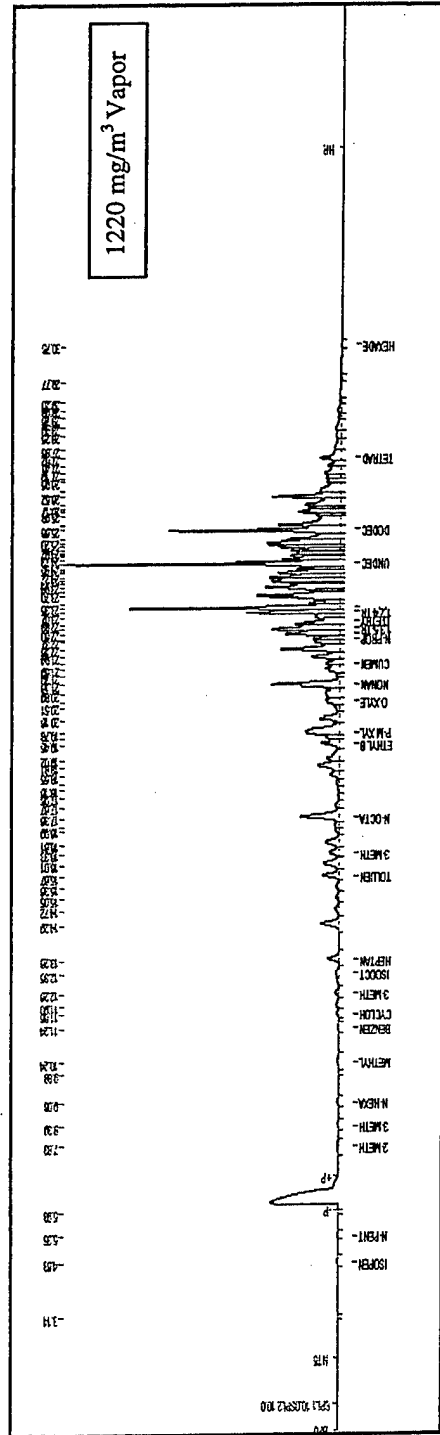
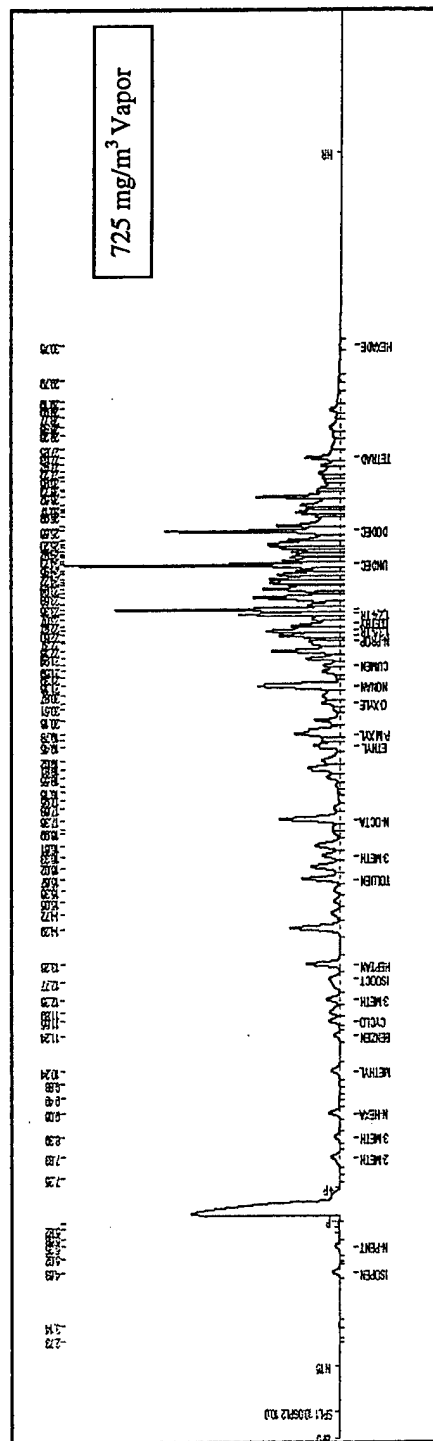
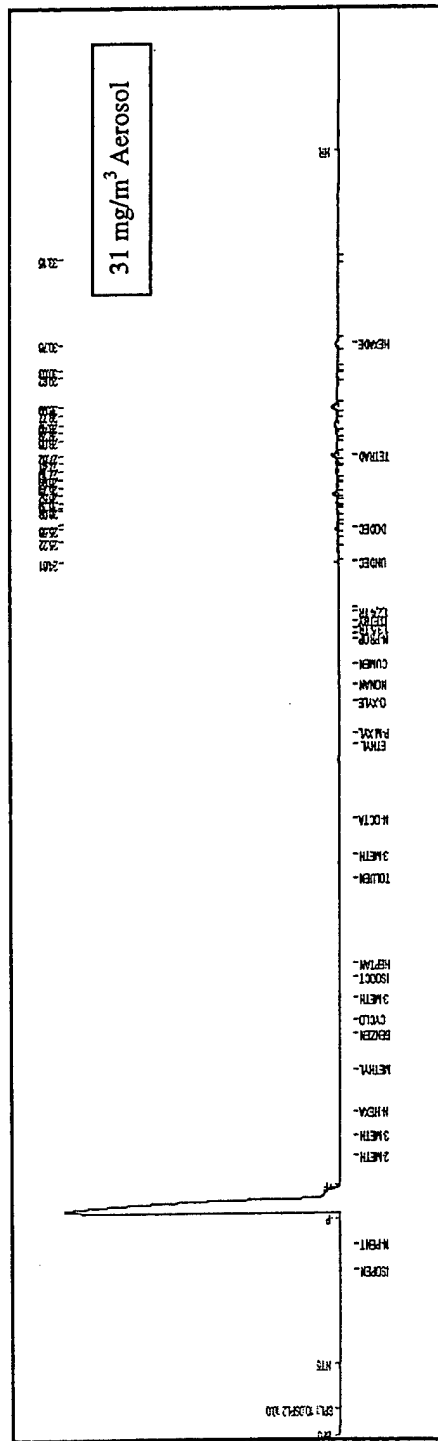


FIGURE 15A - COMPARISON OF CHROMATOGRAMS FOR SELECTED INDIVIDUAL AEROSOL AND VAPOR SAMPLES
JP - 8 + 100 (Mean Concentration 777 mg/m³)



APPENDIX B: TABLE 1B - SUMMARY OF ANALYTICAL DATA
(ADDITIONAL EXPOSURES REPEATED DUE TO MISSING OR INCONSISTENT DATA)

Test Substance	JP 4 (MRD-00-629)				JP 8 (MRD-00-630)		
	Sample #:	1	2	3	1	2	3
Non-Volatile Aerosol (Filter), mg/m ³		0	0	0	1711	1611	1286
Volatile Hydrocarbons (Sorbent tube), mg/m ³		257	0	949	1	1	2331
Total Analytical Concentration, mg/m ³		257	0	949	1711	1611	3617
Mean Analytical Concentration, mg/m ³		402 ± 491			2313 ± 1130		
Nominal Concentration, mg/m ³		1111			7222		

1 - No Sample Taken

TABLE 2B - SUMMARY OF ANIMAL RESPONSE DATA
(ADDITIONAL EXPOSURES REPEATED DUE TO MISSING OR INCONSISTENT DATA)

EXPOSURE CONCENTRATION	ANIMAL NUMBER	BODY WEIGHT (g)	EXPOSURE RESPONSE (%)	RECOVERY RESPONSE (%)	IRRITATION TYPE/ SEVERITY*	GROSS OBSERVATIONS	
						(pretest) (inchamber)	(postdose)
402 mg/m ³	JP - 4						
	IAY956	28	-19	99	SENSORY/SLIGHT	NOA	NOA
	IAY957	29	-21	102	SENSORY/MODERATE	NOA	NOA
	IAY962	29	-24	88	SENSORY/MODERATE	NOA	NOA
	IAY965	29	-28	86	SENSORY/MODERATE	NOA	NOA
	MEAN	29	-23	94			
	S.D.	0.5	3.9	7.9			
2313 mg/m ³	JP - 8						
	IAY991	30	-76	33	SENSORY/EXTREME	NOA	NOA
	IAY988	28	-51	71	SENSORY/EXTREME	NOA	NOA
	IAY992	30	-64	80	SENSORY/EXTREME	NOA	NOA
	IAY993	28	-57	70	SENSORY/EXTREME	NOA	NOA
	MEAN	29	-62	64			
	S.D.	1.2	10.7	20.8			

NOA - NO OBSERVABLE ABNORMALITIES

* Severity categorized as: slight = 12-19%; moderate = 20-49%; extreme = ≥ 50%.

FIGURE 1B - INDIVIDUAL RESPIRATORY RATES
(ADDITIONAL EXPOSURES REPEATED DUE TO MISSING OR INCONSISTENT DATA)

